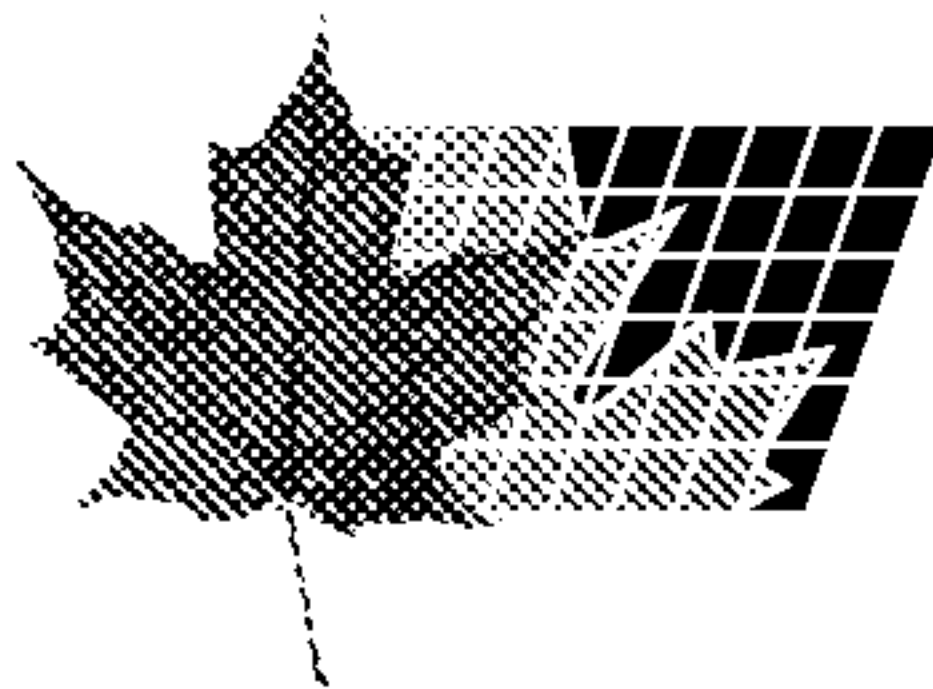




(72) LEMISCHKA, IHOR, US

(72) MOORE, KATERI, US

(71) PRINCETON UNIVERSITY, US

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(54) **GENES REGULANT LES CELLULES SOUCHE**

HEMATOPOIETIQUES ET UTILISATION DE CES GENES

(54) **GENES THAT REGULATE HEMATOPOIETIC BLOOD
FORMING STEM CELLS AND USES THEREOF**

(57) The present invention provides an isolated nucleic acid derived from an isolated hematopoietic stem cell. The present invention additionally provides an isolated hematopoietic stem cell specific protein or a portion thereof encoded by the provided nucleic acid. In addition, the present invention provides a method for generating a stem cell/progenitor cell from a primitive hematopoietic cell. Also, the present invention further provides a method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity. The present invention additionally provides a molecularly defined primitive hematopoietic stem cell. Finally, the present invention provides a method of ex vivo expansion of hematopoietic stem cells.

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(54) Title: GENES THAT REGULATE HEMATOPOIETIC BLOOD FORMING STEM CELLS AND USES THEREOF (57) Abstract The present invention provides an isolated nucleic acid derived from an isolated hematopoietic stem cell. The present invention additionally provides an isolated hematopoietic stem cell specific protein or a portion thereof encoded by the provided nucleic acid. In addition, the present invention provides a method for generating a stem cell/progenitor cell from a primitive hematopoietic cell. Also, the present invention further provides a method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity. The present invention additionally provides a molecularly defined primitive hematopoietic stem cell. Finally, the present invention provides a method of ex vivo expansion of hematopoietic stem cells.		

GENES THAT REGULATE HEMATOPOIETIC BLOOD FORMING STEM CELLS AND USES THEREOF

5

GOVERNMENTAL SUPPORT

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Throughout this application, various publications are referenced by number. Full citations for these publications may be found listed at the end of the specification and preceding the Claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of
15 the art. A Sequence Listing is provided.

FIELD OF THE INVENTION

The present invention relates to hematopoietic stem cells and the stem cell and support cell genes that support stem cell replication and differentiation.
20

BACKGROUND OF THE INVENTION

The adult hematopoietic system is organized as a hierarchy of cells with decreasing self-renewal and multilineage differentiation potential. This is accompanied by progressively larger numbers of more mature cells and an increasing tendency to be in active cell cycle
25 (Lemischka, I.R., 1992; Morrison, S.J., et al. 1995). Collectively, the properties of this hierarchical system result in the balanced, lifelong production of at least eight distinct cell lineages. A population of stem cells establishes the entire hierarchy; therefore, in order to understand the fundamental mechanisms responsible for normal hematopoiesis it is ultimately necessary to understand the biology of the stem cells.

30 Most information concerning the biology of stem cells has been obtained from the mouse model. In this system, the most critical, characteristic property of the stem cell population has been defined; that is, its ability to reconstitute a normal blood system in a transplanted host. A number of variations on the basic transplantation assay have been

described (Harrison, D.E. 1980; Spangrude, G.J., et al. 1995). All of these systems, together with the appropriate donor vs. host or clonotypic markers have rigorously defined the most primitive stem cells and have provided a description of their developmental properties. Perhaps the most striking characteristics of this cell population
5 come from retroviral "marking" studies (Lemischka, I.R. 1992). These studies clearly show that a single stem cell clone is both necessary and sufficient, not only to sustain lifelong, multilineage hematopoiesis in one primary recipient but in numerous secondary animals. This illustrates the remarkable proliferative potential of the stem cell and directly demonstrates stem cell self-renewal. A major advance in mouse stem cell
10 biology was the development of strategies which facilitate the substantial enrichment of stem cell activity (Bauman, J.G., et al. 1988; Spangrude, G.J., et al. 1988; Jordan C.T., et al 1990). Purification procedures enabled the first direct approaches to unravel the mechanisms responsible for the unique biological properties of the stem cell population. A key observation was that the phenotypically defined stem/progenitor cell population is
15 heterogeneous with respect to in vivo functional properties (Fleming, W.G., et al 1993; Li, C.L. and Johnson G.R. 1992; Spangrude, G.J. and Johnson, G.R. 1990, Jones R., et al. 1990; Uchida, N., et al. 1993). In addition to the in vivo repopulating cells, other primitive progenitor cells are often contained in a purified population (Weilbaecher, K., 1991; Trevisan, M. and Iscove, N.N. 1995; Ogawa, M. 1993). These can be assayed in a
20 variety of in vitro systems. Whether all of these in vivo and in vitro activities represent discrete cell subpopulations or whether there is a continuum of functional potential is still an unanswered question. Recent studies have suggested distinct physical properties for functionally different activities within the primitive population Morrison, S.J., and Weissman, I.L. 1994); Morrison, S.J. et al. 1997; Jones, R.M., et al. 1996). A second set
25 of observations revealed an inverse correlation between a tendency for active cell cycling and primitive, uncommitted developmental potential in BM (Spangrude, G.J., and Johnson, G.R. 1990). In fetal liver a higher proportion of primitive stem cells is actively cycling (Fleming, W.H., et al. 1993). Moreover, it has been shown that fetal stem cells are more potent than adult stem cells in LTRA (Jordan, C.T., et al. 1995; Pawliuk, R., et
30 al 1996). These are exciting observations because they suggest that rapid stem cell cycling can be compatible with the maintenance of primitive in vivo activity. Very recent studies suggest that the adult BM stem cell compartment may in fact be cycling at a very slow rate (Bradford, G.B., et al. 1997). Clearly, stem cell cycle regulation is a critical

area for investigation. Stem cell purification has facilitated studies aimed at ex vivo maintenance or expansion of the most primitive, transplantable stem cell. Most culture systems strongly favor a differentiation process (Van der Sluijs, J.P., et al. 1993; Traycoff, C.M., et al. 1996; Peters, S.O., et al. 1995; Knobel, K.M., et al. 1994).
5 However, several recent reports have been encouraging. It has been shown that colonies grown in defined cytokines can retain not only myeloid and erythroid but also lymphoid potentials (Ball, T.C., et al. 1995). Moreover, the short-term (2-3 weeks) maintenance of LTRA has been demonstrated in suspension cultures supported by IL6, IL11, together with ckit ligand (KL) or flk2/flt3 ligand (FL) (Yonemura, Y.H., et al. 1997). A recent
10 report has shown that colonies initiated in cytokine-supplemented semisolid cultures retain LTRA (Trevisan, M., et al. 1996). The studies described herein have developed a stromal cell line supported system which quantitatively maintains LTRA for an extended (4-7 weeks) time (Moore, F.A., et al. 1997).

In the human system it is clearly not possible to do the same kind of extensive
15 marking and transplantation assays. However, several xenograft model systems have been developed to assess the in vivo behavior of human stem cells (Traycoff, C., et al. 1994; Turner, C., et al. 1996; Cashman, J., et al. 1997). Some of these experiments can be done quantitatively in limiting dilution (Bhatia, M., et al. 1997). A very recent study has demonstrated a common proviral integration site in granulocyte macrophages and T-
20 cells derived from beige/nude/XID mice 7-11 months after engraftment with genetically transduced human stem cells (Nolta, J.A., et al. 1996). This important study paves the way for precise in vivo clonal analyses. The largest amount of functional information about human stem/progenitor cells has been obtained in vitro using a wide range of stromal cell and cytokine supported culture systems. It is not possible herein to describe
25 and properly accredit all of the important studies, however several advances deserve mention. The long-term culture-initiating cell (LTCIC) assay measures the in vitro production of colony forming cells (CFC) after periods of at least five weeks in culture (Sutherland, H.S., et al. 1989). The cells producing these CFC derive from a population of cells which, at least to some extent, probably overlaps with the most primitive
30 compartment. The maintenance and expansion of primitive functional abilities in this culture system has recently been documented (Petzer, A.L., et al. 1996). A variation on this assay system, the extended LTCIC (ELTCIC) has been suggested to measure an even more primitive cell population in BM and CB (Hao, Q.L., et al. 1996). A very exciting

prospect for the near future will be the integration of the various in vivo xenograft assays with the in vitro LTCIC and ELTCIC systems. Some very recent efforts have suggested that the NODSCID xenograft system and the LTCIC assay may measure distinct stem/progenitor subsets (Larochelle, A.J., et al. 1997). Clearly, much more work needs to be done, however, it may be anticipated that the ELTCIC system will provide the “bridge” in this continuum. Collectively, and including the various strictly cytokine-driven systems, the above studies illustrate the current possibility to accurately and quantitatively reveal the majority (if not all) functional entities in the human stem/progenitor cell hierarchy. The physical characterization and purification of human stem/progenitor cells has proceeded along lines which are parallel to the mouse system. Indeed, because of clinical impetus, it can be argued that they are further advanced. Thus, as measured in the range of assays discussed above, the consensus physical phenotype of the most primitive portion of the human stem/progenitor hierarchy is CD34+Lin-CD38- (Terstappen, L.W.M.M., et al. 1991). The CD34+Lin-CD38+ subset contains less primitive, more committed cellular entities. Other studies have shown that, similar to the mouse, low level expression of Thy1 (CD90) is a feature of the primitive human stem cell (CD34+Lin-CD90+)(Baum, C.M., et al. 1992; Craig, W., et al. 1993). Most CD90+ cells in this compartment are CD38-. Therefore, the consensus phenotype can be described as CD34+Lin-CD90+ (Craig, W., et al. 1993). Two potential differences with the murine system can now be highlighted. First, a very recent and elegant study has shown that the most primitive mouse stem cell may in fact be CD34-/lo (Osawa, M., et al. 1996) . Whether this is a genuine difference or whether it reflects the ability to perform more accurate long-term engraftment studies in the mouse remains to be determined. Second, it has been suggested that in the mouse, CD38 expression is a positive indicator for primitive stem cell function in a purified population (Randall, T.D., et al. 1996) . As in the mouse, human stem/progenitor cells have been identified and purified from various sources. These include: adult BM (Baum, C.M., et al. 1992), CB (DiGiusto, D.L., et al 1996) , fetal liver (Craig, W., et al. 1993) and peripheral blood stem cells after various mobilization protocols (Murray, L., et al. 1994). Similar to the data obtained in the murine system, comparative studies reveal that, in general, the basic and fundamental functional properties of stem/progenitor cells are shared regardless of the tissue source. There are however, significant functional and physical differences.

Numerous insights into hematopoietic molecular control mechanisms have come from gene-targeting studies in mice. Mutations in specific genes, most notably, those encoding transcription or DNA-binding proteins, have profound cell-intrinsic, global or lineage-specific effects on hematopoietic development (Shivdasani, R.A., and Orkin, S.H. 1996; Orkin, S.H. 1996). In the latter cases, it is tempting to speculate that the phenotypes result from defects in the commitment process. However, malfunctions in the commitment decision to "set up" a program of differentiation are difficult to distinguish from malfunctions in the differentiation program itself. Two gene products, AML1 (CBF2) and SCL (tal-1) appear to be necessary global regulators of hematopoiesis (Wang, W., et al. 1996; Okuda, T., et al. 1996; Porcher, C., et al. 1996; Robb, L., et al. 1996). Whether these molecules act to specify a hematopoietic stem cell or by other means is an open question. Interestingly, both of these molecules play roles in leukemic transformation. A very important gain of function study documents the apparent ability of HOXB4 to increase primitive cell numbers without significant impairment of differentiation abilities (Sauvageau, G., et al. 1995). Together with observations that HOXA9 is translocated in myeloid leukemia (Nakamura, T., et al. 1996; Borrow, J., et al. 1996), these studies suggest an important hematopoietic role for homeobox proteins. Without question, the above and other studies have identified important regulators of hematopoiesis. However, in almost all cases these regulators were first identified in other systems. The opposite approach is to directly search for stem cell regulators in stem cells, Graf, L., and Torok-Storb, B. 1995; Yang, Y., et al. 1996). The present invention solves these problems.

SUMMARY OF THE INVENTION

The human hematopoietic stem/progenitor cell population has been extensively characterized according to physical and antigenic criteria as well as in a variety of in vitro and in vivo assay systems. Collectively the human studies have revealed similarities to the hierarchical stem/progenitor cell organization defined in the murine system. In spite of significant strides in the identification of cytokines which can act on stem cells, it has not been possible to define a system where undifferentiated expansion of the most primitive stem cell population occurs. Similarly, it has not been possible to direct differentiation along lineage-specific pathways. These limitations, which also apply to the murine system, have hampered the elucidation of regulatory mechanisms which

mediate the most fundamental aspect of stem cell biology; that is, the decision to self-renew or commit to differentiation. As a consequence, very little is known about the molecular biology of the most primitive hematopoietic stem cell in any organism. It was hypothesized that the regulation of primitive stem cells will be mediated at least in part by the products of genes which are uniquely or predominantly expressed in these cells. One precedent for an important, differentially-expressed molecule is the flk2/flt3 receptor tyrosine kinase. There presumably are other important and differentially expressed gene products. Therefore, it is an object of the present invention to identify these molecules and address their functional roles. Specifically, an aspect of identification of gene expression patterns specific to primitive human stem cells is the molecular phenotype of the human stem cell. The present invention describes methods to define the profile of genes specifically expressed in undifferentiated human stem/progenitor cell populations.

A primary focus of the present invention is on primitive cells isolated from normal bone marrow (BM) samples. The present invention further comprehends use of other sources of stem cells, such as umbilical cord blood (CB).

The methods of the present invention combine diverse technical approaches and sophisticated bioinformatic analyses.

This invention further provides methods to identify genes whose expression can be modulated by cytokine or stromal-dependent culture and/or by cell-cycle status.

Another object of the present invention is to provide methods for the functional characterization of human stem cell-specific gene products. An aspect of this invention is a method to facilitate the functional characterization of specifically expressed gene products as candidate regulators of a variety of stem/progenitor cell processes. In particular, a provided method uses an in vitro system which approximates many characteristic properties of normal stem cells to analyze positive and negative regulation of proliferation, cell-cycle parameters, apoptosis and commitment.

It is a further goal of the present invention to provide a necessary (and usually missing) component for stem cell gene-expression screens; that is, the ability to quickly assess the function of extensive panels of genes.

It is also an object of the present invention to provide a method for the functional identification of stem cell regulators. An aspect of this invention is a facile screening method for "categorizing" large populations of specifically-expressed molecules according to their potential roles in a variety of stem/progenitor cell processes. Gain of

function as well as loss of function approaches are contemplated by the present invention. This method does not rely on any prior knowledge of nucleotide or predicted protein sequence.

Accordingly, the present invention provides an isolated nucleic acid
5 derived from an isolated hematopoietic stem cell, the isolated nucleic acid comprising the following characteristics: (1) specifically expressed in the hematopoietic stem cell; and (2) encoding a hematopoietic stem cell – specific protein.

The present invention additionally provides an isolated hematopoietic stem cell
10 specific protein or a portion thereof encoded by the provided nucleic acid. The present invention further still provides a nucleic acid probe capable of specifically hybridizing with the provided nucleic acid under standard hybridization conditions.

Also, the present invention provides an antibody capable of specifically binding to the provided protein without substantially cross-reacting with a non-stem cell specific
15 protein or homologs thereof under conditions permissive to antibody binding. Additionally, the present invention provides a cell capable of producing the provided antibody.

In addition, the present invention provides a method for identifying the presence of a primitive hematopoietic stem cell in a sample comprising nucleic acids specifically
20 expressed in hematopoietic stem cells. Further still, the present invention provides a method for generating a stem cell/progenitor cell from a primitive hematopoietic cell in a sample.

The present invention further provides a method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity.

25 The present invention even further still provides a method for identifying primitive hematopoietic stem cell-specific nucleic acids.

Also the present invention additionally provides a molecularly defined primitive hematopoietic stem cell.

Yet additionally, the present invention provides a method for treating a condition
30 in a subject comprising administering to the subject a therapeutically effective amount of a provided pharmaceutical composition. The present invention provides a method of introducing an exogenous nucleic acid into a hematopoietic stem cell.

Finally, the present invention provides a method of ex vivo expansion of hematopoietic stem cells. The expanded cells are available to receive exogenous genes, including by retroviral or other vectors which require a round of replication. Alternatively, the cells are available for transplantation either autologously or heterologously.

5

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1. Schematic "flow of information" strategy.

10

FIGURE 2. Mammalian stem cell system (black circle = strong hybridization signal, gray circle = detectable hybridization signal, and open circle = no detectable hybridization signal). The Smc-34 cDNA is a completely novel sequence with a predicted leucine zipper and several potential membrane spanning domains.

15

FIGURES 3A-3B. (right panel), the control, non-subtracted RDA cDNA population (38-) contains b-actin sequences which are missing in the two subtracted RDA populations, 38- and 38-)38+. A differentially expressed gene (HDD-2, described below) is enriched in the 38- RDA population and at least retained in the 38-)38+ RDA population (Figure 3A, left panel). Two, bi-directional, RDA cDNA populations (38- and the converse 34-)38) were used to probe (See Figure 3) duplicate arrays of a subtracted 38- library (Figure 3B).

20

FIGURE 4A-4E. **Figure 4A:** 34B4 (SEQ.ID.No.: 69) is closely related to a gene encoding TINUR. The sequence homologies and restricted expression pattern of 34A5 is shown in **Figures 4B and 4C**. In **Figure 4C and 4D** (and also 5a, 5b, and 6b) there are twenty-one samples of capfinder-amplified cDNA from various hematopoietic populations. From left to right these are: four CD34+Lin- populations, three CD34+Lin-CD90+ populations, two CD34- populations, four CD34+Lin-CD38+ (obtained from the same BMs as the CD38- samples in lanes 1-4), two CD34+Lin- samples (obtained from the same BMs as the CD90+ samples in lanes 5 and 6), three CD34+Lin- populations obtained after 1, 2 or 4 days of culture and finally their three CD34+Lin-CD38+ counterparts. The 38G2 cDNA (SEQ.ID.No.: 70) is closely homologous to the

30

LTG9/MLLT3 gene located on 9q22 and involved in t(9;11) leukemia (Iida, S., et al. 1993) (Figure 4E).

FIGURES 5A-5B. G0S3, a fos-related gene (Heximer, S., et al. 1996) (Figure 5A) and
5 (2) HLA-DR (Figure 5B).

FIGURES 6A-6D. Figure 6A. HDD-2 is about 500 bp. It contains a short open reading frame of 89 amino acids (SEQ.ID.No.: 71). The predicted peptide sequence is shown. Figure 6B. The expression profile of HDD-2 demonstrates that it is stem cell restricted.
10 Figure 6C. HDD-2 hybridization to a dot blot with numerous human pA+ mRNA samples (Clontech). HDD-2 hybridization is only visible in kidney (the other "spots" are background). Figure 6D. Genomic Southern blot confirmed that HDD-2 corresponds to a single-copy human gene.

15 **FIGURE 7.** Immunoprecipitation analysis of protein extracts using rat IgG2b isotype control antibody (IgG) or AA4.1 mAb. Protein extracts were prepared from D2N cells; M2.4 cells; AA4-depleted fetal liver cells (FLAA4-); AA4-enriched fetal liver cells (FLAA4+); AA4-depleted bone marrow cells (BMAA4-); AA4-enriched bone marrow cells (BMAA4+). Indicated on the right are positions of molecular weight markers.

20

FIGURES 8A-8C. AA4 expression in retrovirus infected cells. **Figure 8A.** Flow cytometry analysis of NIH 3T3 and EML Cl cells using PE-conjugated AA4.1 antibody before and after infection with REBNA/AA4. **Figure 8B.** Immunoprecipitation of biotin-labeled surface proteins using rat IgG2b isotype control antibody (lanes 1, 3, 5) or
25 AA4.1 mAb (lanes 2, 4, 6). Cellular extracts were prepared from D2N cells (lanes 1 and 2), REBNA/AA4 infected NIH 3T3 fibroblasts (lanes 3 and 4), and REBNA/AA4 infected EML Cl cells (lanes 5 and 6). **Figure 8C.** Immunoprecipitation of cellular extracts using AA4.1. REBNA/AA4 NIH 3T3 cells (lanes 2 -- 4) and REBNA/AA4 EML Cl cells (lanes 6 -- 8) were labeled with 35S-methionine and chased with nonradioactive
30 media for 10 min. (lanes 3 and 7) or 20 min. (lanes 4 and 8). REBNA/GFP NIH 3T3 (lane 1) and REBNA/GFP EML Cl cells (lane 5) are shown as controls. Indicated on the right are positions of molecular weight markers.

FIGURE 9. Nucleotide sequence (SEQ.ID.No.: 72) and the deduced amino acid sequence of AA4 (SEQ.ID.No.: 73). Amino acid sequences is numbered 1 with initiator methionine. Underlined is the putative signal peptide in AA4. The transmembrane domain is double underlined. Light grey line indicates C-type lectin carbohydrate recognition domain. Dark grey lines show EGF-like repeats, while broken grey lines indicate calcium-binding EGF motifs.

FIGURES 10A-10B. Comparison of the primary structures of AA4 and human ClqR.

Figure 10A. Alignment of amino acid sequences of AA4 (SEQ.ID.No.: 73) and ClqR.

Figure 10B. Comparison of the domain structures of AA4 and ClqR. The proteins contain N-terminal signal peptides (SP), a C-type lectin recognition domain, six EGF-like domains, including three calcium-binding (cb) EGF-like domains, and a transmembrane domain (TM).

FIGURE 11. In normal tissues and transformed cell lines, a 7kb RNA species hybridizes with the cloned cDNA (Figures 11A and 11B). In addition to the 7kb species, poly(A)-RNA from D2N cells contains a minor band corresponding to a 3.2 kb mRNA (Figure 11A, lane 8).

FIGURES 12A-12B. Northern blot analysis of AA4 expression in transformed cell lines **Figure 12A** and normal mouse tissues **Figure 12B**. Indicated on the left are positions of 9.44 - 0.24 kb RNA molecular weight markers. Hybridization with D2N poly(A)+ RNA is shown after a 2 hr and overnight exposures. Hybridizations with GAPDH are shown as controls for equal loading.

25

FIGURES 13A-13C. RT-PCR of cDNAs prepared from murine fetal liver, (A) bone marrow-derived hematopoietic cells (B), or differentiating ES cells (C). **Figure 13A.** Lanes 1 and 2, AA4- cells; lane 3, AA4+ cells; lane 4, AA4+Sca-1+c-Kit+Lin^{lo} cells; lane 5, AA4+Sca-1-c-Kit+Lin^{lo} cells. **Figure 13B.** Lane 1, Rho^{lo}Sca-1+Thy-1^{lo}Lin^{lo} cells; lane 2, Rho^{hi}Sca-1+Thy-1^{lo}Lin^{lo} cells; lane 3, Sca-1+Thy-1^{lo}Lin^{lo} cells; lane 4, Lin⁺ cells. **Figure 13C.** ESO, undifferentiated ES cells; BL1 and 2, blast cell colonies; ENT1 and 2, differentiated endothelial cells; HMT1, 2 and 3, differentiated hematopoietic cells. The cDNAs probes used for hybridization are indicated on the right.

30

FIGURE 14. AFT024 maintains *in vivo* repopulating stem cells. The ability of 3 different stromal cell lines to support highly purified fetal liver stem cells was studied. Freshly purified day 14 fetal liver cells were transplanted directly (10^3 AA4.1⁺, lin^{-lo}, Sca-1⁺, c-kit⁺ cells plus 10^6 Ly5.1 competitor BM per mouse; n=6) into Ly5.1 congenic mice (Control). Additional stem cells from the same purification were also used to initiate Dexter-LTC over irradiated AFT024, 2012, and 2018 stromal cell monolayers (10^4 cells/10 cm dish). After 4 weeks of culture, 10% aliquots of each culture (10^3 stem cell equivalents) were transplanted into Ly5.1 recipients (n=8) together with 10^6 competitor Ly5.1 BM cells. Peripheral blood cells from mice were analyzed for the presence of Ly5.2⁺ cells at 5, 12, 24, and 56 weeks after transplant. Error bars represent SEM.

FIGURE 15. Long-term culture on AFT024 maintains greater levels of repopulating stem cell activity than short-term cytokine- or short-term AFT024-supported cultures. The levels of stem cell activity maintained in short-term cytokine-supported and short-term AFT024-supported cultures were compared to those maintained in long-term AFT024 coculture. Purified fetal liver cells were cultured for 5 days in suspension with cytokines or on an AFT024 monolayer (3000/well-12-well tray). Additional cells from the same purification were seeded onto AFT024 monolayers (3000/6 cm dish) and maintained in Dexter-LTC for 5 weeks. At completion of both culture periods the cells were harvested, mixed with Ly5.1 BM and used to transplant mice. Each mouse received 20% of each culture (600 stem cell equivalents) and 4×10^5 competitor BM cells (4 mice/culture). Peripheral blood cells from mice were analyzed for the presence of Ly5.2⁺ cells at 15 weeks after transplant. FL 1.0 ± 0.57 ; FL/SL 0.75 ± 0.25 ; FL/IL-6 1.8 ± 0.14 ; SL/IL-6 3.2 ± 0.46 ; FL/IL-6/SL 1.7 ± 0.21 ; AFT024 5 days 2.8 ± 0.11 ; AFT024 5 weeks 32.2 ± 7.4 * ($p < 0.004$) Students t-test. See Methods for culture conditions and cytokine concentrations. FL, flk2/flt3-ligand; SL, steel factor. Error bars represent SEM.

FIGURE 16. Time course of cobblestone area formation on AFT024. The formation of stromal dependent CA derived from purified fetal liver stem cells was studied in AFT024 cocultures. Characteristic clusters of at least 50 cells were scored as CA over 28 days of culture. Results are expressed as the mean number of CA/1000 input stem cells from 3

separate fetal liver purifications (300-600 cells/well in 12-well trays). Error bars represent the SEM. The frequency of CA after 28 days is approximately 1 for every 20 input stem cells.

- 5 **FIGURE 17.** High-proliferative potential multilineage clonogenic progenitors are selectively expanded on AFT024. The clonogenic progenitor content of stem cells maintained in AFT024 supported Dexter-LTC was determined. Enriched fetal liver stem cells were seeded onto AFT024 monolayers, at various time points, an individual well was harvested and the cells placed into semi-solid clonogenic progenitor assay (CFU-C).
 10 Colonies were scored at 8-14 days. Colonies were designated as HPP upon reaching a size ≥ 1 mm after 8 days. CFU numbers at days 0, 4, and 28 are averaged from 3-5 individual stem cell purifications. Error bars represent the SEM for these experiments. Other time points are individual determinations. CFU are normalized to 1000 input stem cells in the stromal cocultures for comparison to day 0 progenitors. *CFU-Mix ($p=.01$)
 15 and *CFU-HPP-Mix ($p=.001$) are significantly expanded at day 28 compared to day 0, (Student's T-test).

- FIGURES 18A-18B.** Cobblestone area-initiating cells are expanded on AFT024. **Figure 18A.** A quantitative estimate of the number of 28 day cultured stem cell
 20 equivalents required to form a CA after replating on secondary AFT024 monolayers was determined. Four different AFT024 cocultures from separate fetal liver purifications were studied (A, B, C, and D). In limiting-dilution assay, the frequency of stem cell equivalents required to form a CA in another 7 days was 1 in 4 (3.56 ± 0.64 , $r^2=0.96$). **Figure 18 B.** CA maintenance in one of the 4 above cultures was followed for an
 25 additional 4 weeks. The frequency of stem cell equivalents maintaining CA was determined. At 37% negative wells the frequencies were: 2 weeks 1 in 3, 3 weeks 1 in 10, 4 weeks 1 in 19, and at 5 weeks 1 in 29.

- FIGURES 19A-19B.** Dlk expression analysis in stromal cell lines. **Figure 19A.** (Top) A
 30 1.6 kb dlk transcript is visualized in the parental AFT024 and 2012 cell lines and their subclones, but not in 2018, CFC034 and BFC012. (Bottom) The same filter hybridized with a b-actin probe. **Figure 19B.** RT-PCR analysis of 14 fetal liver-derived stromal cell lines and other cell lines.

FIGURE 20. Cobblestone area formation by hematopoietic stem cells in the presence of soluble dlk protein. Data are from 4 experiments; 2 each with adult BM cells (Sca-1⁺, c-kit⁺, lin^{lo/-}) and day 14 fetal liver cells (AA4.1⁺, Sca-1⁺, c-kit⁺, lin^{lo/-}). Results are expressed as the ratio/fold-increase in CSA number for fourteen data points each (bars represent, none vs control; dlk vs none; dlk vs control) for the 4 different experiments. Error bars represent SEM. *P= 0.01 comparing dlk vs none to none vs control, **P= 0.001 comparing dlk vs control to none vs control (Student's t-test).

FIGURE 21. Membrane bound dlk expression in transfected BFC012 cells. Full length dlk cDNA was transfected into BFC012 cells. (Left) A flow diagram of dlk expression in transfected BFC012 populations (BFC-dlk) and cells transfected with the selection plasmid alone (BFC-Zeo). (Right) Expression of dlk in a cloned line (BFC-dlk-5) derived from the expressing population and a control clone (BFC-Zeo-1).

FIGURES 22A-22B. CSA formation by hematopoietic stem cells in the presence of membrane-bound dlk. **Figure 22A.** Bars labeled BFC are from 5 groups (nontransfected BFC012 cells, two control pZeo transfected BFC012 populations, and two clones derived from the pZeo transfected populations). Bars labeled BFC-dlk are from three groups shown to express transfected dlk; one dlk-transfected BFC012 population (BFC-dlk) and two individual transfected clones (BFC-dlk-1 and BFC-dlk-5). Error bars represent SEM. ** P< 0.001 days 3, 4, and 5; *P< 0.01 days 6 and 7 (Student's t-test). **Figure 22B.** A clone derived from the dlk transfected populations of BFC012 cells (BFC-dlk-5) and a clone derived from pZeo transfected populations (BFC-Zeo-1) were used for CSA assay with purified fetal liver stem cells. CSA/1000 input stem cells are expressed as the mean of three individual experiments, error bars represent the SEM. **P< 0.001 at days 4, 6, and 8 (Student's t-test).

FIGURES 23A-23B. HPP multilineage clonogenic progenitors and *in vivo* repopulating stem cells are maintained in short-term dlk-expressing cocultures. **Figure 23A.** Fetal liver stem cells were purified as described and assayed for their progenitor content immediately after purification and after culture on BFC-dlk-5, and BFC-Zeo-1. At day 4 the cultures were used for clonogenic progenitor (3 experiments) and transplantation assay (2 experiments). Bars represent data from 3 experiments with day 0 cells (Fresh) and day 4

cocultured cells (BFC-dlk-5 and BFC-Zeo-1), error bars represent SEM. *P= 0.01 for total CFU-C from fresh stem cells compared to total CFU-C from BFC-dlk-5 cocultures at day 4; ** P= 0.001 for total CFU-C from BFC-dlk-5 compared to total CFU-C from BFC-Zeo-1 (Student's t-test). **Figure 23 B.** Analysis of *in vivo* repopulating ability of purified fetal liver stem cells cocultured for 4 days on BFC-dlk-5, BFC-Zeo-1, and AFT024 monolayers. Results are from nine individual mice in two experiments (4-5 mice in each experiment) at 10 weeks after transplantation. P= 0.05 for BFC-dlk-5 vs BFC-Zeo-1 (Student's t-test).

FIGURE 24. Genes and predicted proteins isolated from primitive stem cells by the techniques of the present invention. (Sequence Identification Numbers are indicated on the figure).

DETAILED DESCRIPTION

The present invention provides an isolated nucleic acid derived from an isolated hematopoietic stem cell, the isolated nucleic acid comprising the following characteristics: (1) specifically expressed in the hematopoietic stem cell; and (2) encoding a hematopoietic stem cell – specific protein.

An embodiment of this invention further comprises the following characteristic:
capable of hybridizing under standard conditions with a sequence selected from the group consisting of SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25, SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39, SEQ.ID.No.: 40, SEQ.ID.No.: 41, SEQ.ID.No.: 42, and SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a portion thereof. A portion thereof, in a preferred embodiment of this invention is the 5' end region or the 3' end region of the nucleic acid.

In another preferred embodiment, a portion thereof is at least a 8-18 nucleotide portion of the coding region. In yet another preferred embodiment, a portion thereof is at least a 8-18 nucleotide portion of a non-coding regulatory region or a binding region such as a stem-cell specific promoter or enhancer region. According to still another embodiment of the provided isolated nucleic acid further comprises the characteristic of encoding a protein capable of modulating hematopoietic stem cell activity. According to this invention modulating hematopoietic stem cell activity includes up-regulating, down-regulating or otherwise changing the activity of the hematopoietic stem cell. Such activity is contemplated as inducing differentiation or inhibiting differentiation of the cell. However, directing differentiation toward one or another daughter cell type is also within the scope of a preferred embodiment of this invention. Other preferred embodiments include but are not limited to modulation of transcription, translation, gene splicing, transport, proteolytic processing, replication, expression of cell surface markers and transplantation. According to still another embodiment of the present invention, the activity is selected from the group consisting of hematopoietic stem cell differentiation and hematopoietic stem cell replication. According to yet another embodiment of this invention, the protein is selected from the group consisting of a growth factor, a transcription factor, a splicing factor, a capping factor, a transport protein, a translation factor, and a replication factor. In one preferred embodiment of this invention, the provided nucleic acid comprises the nucleotide sequence of SEQ.ID.No.: 72, an analog thereof, or a portion thereof. According to another preferred embodiment of this invention, the hematopoietic stem cell is a primitive hematopoietic stem cell. In one embodiment of this invention, the primitive hematopoietic stem cell is selected from the group consisting of an umbilical cord cell, a bone marrow cell and a fetal liver cell. In a preferred embodiment of this invention, the primitive hematopoietic stem cell is selected from the group consisting of a AFT024 cell, a 2012 cell and a 2018 cell.

The present invention further provides a composition comprising the provided nucleic acid, wherein the nucleic acid comprises one selected from the group consisting of SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25,

SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39, SEQ.ID.No.: 40, SEQ.ID.No.: 41, SEQ.ID.No.: 42, SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a portion thereof. According to one embodiment of this invention, the the nucleic acid is selected from the group consisting of DNA, RNA and cDNA. Another embodiment of this invention is a vector comprising the provided nucleic acid. According to yet another embodiment, the vector comprises viral or plasmid DNA. A further embodiment of this invention is an expression vector comprising the provided nucleic acid and a regulatory element. A still further embodiment of this invention is a host vector system which comprises the expression vector in a suitable host. In a preferred embodiment of this invention, the suitable host is selected from the group consisting of a bacterial cell, a eukaryotic cell, a mammalian cell and an insect cell.

The present invention additionally provides an isolated hematopoietic stem cell specific protein or a portion thereof encoded by the provided nucleic acid. According to one embodiment of this invention is the provided protein further comprising the characteristic of being capable of modulating hematopoietic stem cell activity. According to this invention modulating hematopoietic stem cell activity includes up-regulating, down-regulating or otherwise changing the activity of the hematopoietic stem cell. Such activity is contemplated as inducing differentiation or inhibiting differentiation of the cell. However, directing differentiation toward one or another daughter cell type is also within the scope of a preferred embodiment of this invention. Other preferred embodiments include but are not limited to modulation of transcription, translation, gene splicing, transport, proteolytic processing, replication, expression of cell surface markers and transplantation. According to still another embodiment of the present invention, the activity is selected from the group consisting of hematopoietic stem cell differentiation and hematopoietic stem cell replication. According to yet another embodiment of this invention, the protein is selected from the group consisting of a growth factor, a transcription factor, a splicing factor, a capping factor, a transport protein, a translation factor, and a replication factor. According to one embodiment the activity is selected from the group consisting of hematopoietic stem cell differentiation and hematopoietic

stem cell replication. According to another embodiment, the protein is selected from the group consisting of a growth factor, a transcription factor, a splicing factor, a capping factor, a transport protein, a translation factor, and a replication factor. According to still another embodiment, the protein has substantially the same amino acid sequence as one
5 selected from the group consisting of SEQ.ID.No.: 42, SEQ.ID.No.: 44, SEQ.ID.No.: 46, SEQ.ID.No.: 48, SEQ.ID.No.: 50, SEQ.ID.No.: 52, SEQ.ID.No.: 54, SEQ.ID.No.: 56, SEQ.ID.No.: 58, SEQ.ID.No.: 60, SEQ.ID.No.: 62, SEQ.ID.No.: 64, SEQ.ID.No.: 66, SEQ.ID.No.: 68, SEQ.ID.No.: 70, SEQ.ID.No.: 71, and SEQ.ID.No.: 73.

The present invention further still provides a nucleic acid probe capable of
10 specifically hybridizing with the provided nucleic acid under standard hybridization conditions.

Also, the present invention provides an antibody capable of specifically binding to the provided protein without substantially cross-reacting with a non-stem cell specific protein or homologs thereof under conditions permissive to antibody binding.
15 Additionally, the present invention provides a cell capable of producing the provided antibody.

In addition, the present invention provides a method for identifying the presence of a primitive hemopoietic stem cell in a sample comprising nucleic acids specifically expressed in hematopoietic stem cells comprising (a) contacting the sample with the
20 provided antibody under conditions permissive to the formation of an antibody complex; (b) detecting the presence of the complex formed in step (a), the presence of a complex formed indicating the presence of a primitive hemopoietic stem cell in the sample. According to one embodiment of this invention, the antibody is labeled with a detectable marker. In a preferred embodiment, the detectable marker is selected from the group
25 consisting of a radioactiveisotope, enzyme, magnetic bead, dye, flourescent marker and biotin.

Further still, the present invention provides a method for generating a stem cell/progenitor cell from a primitive hematopoietic cell in a sample comprising contacting the sample with the provided protein . Another embodiment of this invention, provides a
30 method for generating a stem cell/progenitor cell from a primitive hematopoietic cell in a sample comprising contacting the sample with the provided nucleic acid. According to an preferred embodiment, the nucleic acid is in an expression vector. According to another preferred embodiment the nucleic acid is introduced into the cell under conditions

permissive to the expression of the nucleic acid.

The present invention further provides a method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity comprising: (a) contacting the hematopoietic stem cell with the sample; (b) determining the
5 hematopoietic stem cell activity; and (c) comparing the hematopoietic stem cell activity determined in step (b) with the activity determined in the absence of the compound an increase or decrease in hematopoietic stem cell activity indicating the presence in the sample of a compound that modulates hematopoietic stem cell activity. According to one embodiment, the activity is selected from the group consisting of gene expression,
10 replication, differentiation, transplantation, and self regeneration. The present invention also still further provides a compound identified by the method of this invention, previously unknown.

The present invention even further still provides a method for identifying primitive hematopoietic stem cell-specific nucleic acids , comprising: (a) creating a
15 primitive hematopoietic stem cell cDNA library and a non-primitive stem cell immune cell cDNA library; and (b) subtracting the two libraries, thereby identifying primitive stem cell specific nucleic acids. According to one embodiment is (i) contacting the nucleic acids of the stem cell and non-stem cell libraries with each other under conditions permissive to hybridization, thereby forming hybrid complexes; (ii) separating the hybrid
20 complexes formed in step (b) from the nucleic acids which did not form complexes; and (iii) isolating the nucleic acids which did not form complexes, thereby identifying hematopoietic stem cell specific nucleic acids. In still another embodiment, step (ii) further comprising amplification of the nucleic acids. Yet another embodiment is step (iii) further comprising amplification of the nucleic acids which did not form complexes.
25 Even still another embodiment is further comprising displaying the amplified DNA on a chromatography gel. A further embodiment is step (b) comprising differential display of the two libraries, thereby identifying primitive stem cell specific nucleic acids. Also yet another embodiment is step (b) comprising representation difference analysis of the two libraries, thereby identifying primitive stem cell specific nucleic acids. Yet even another
30 embodiment is further comprising cloning the stem cell specific nucleic acid. According to a preferred embodiment, the stem cell is selected from the group consisting of AF024, 2012, and 2018. The present invention further provides a nucleic acid identified by the provided method.

The present invention additionally provides a composition comprising the provided compound and a carrier. An embodiment of this invention is a pharmaceutical composition comprising the provided compound and a pharmaceutically acceptable carrier.

5 Also the present invention additionally provides a primitive hematopoietic stem cell specifically expressing one selected from the group consisting of: SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, 10 SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25, SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39, SEQ.ID.No.: 40, SEQ.ID.No.: 41, 15 SEQ.ID.No.: 42, and SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a portion thereof. An embodiment of this invention is a primitive hematopoietic stem cell specifically expressing a nucleic acid identified by the provided method.

20 Yet additionally, the present invention provides a method for treating a condition in a subject comprising administering to the subject a therapeutically effective amount of the provided pharmaceutical composition. According to an embodiment of this invention, the condition is an immune system condition. In a further embodiment of this invention, the condition is leukemia.

25 The present invention provides a method of introducing an exogenous nucleic acid into a hematopoietic stem cell comprising contacting the stem cell with the provided composition.

Finally, the present invention provides a method of ex vivo expansion of hematopoietic stem cells comprising contacting the cell with the provided composition. 30 According to an embodiment of this invention, the ex vivo expanded hematopoietic stem cells are available for therapeutic use. The expanded cells are available to receive exogenous genes, including by retroviral or other vectors which require a round of

replication. Alternatively, the cells are available for transplantation either autologously or heterologously.

As used herein, the term, a sequence is conserved if there is substantial homology of sequence between multiple gene species.

5 As used herein, the terms, "hybridization" and "binding" in the context of probes, primers and denatured DNA are used interchangeably. Probes which are hybridized or bound to denatured DNA are aggregated to complementary sequences in the polynucleotide. Whether or not a particular probe remains aggregated with the polynucleotide depends on the degree of complementarity, the length of the probe, and
10 the stringency of the binding conditions. The higher the stringency, the higher must the degree of complementarity, and/or the longer the probe.

As used herein, the term, probe, refers to an oligonucleotide designed to be sufficiently complementary to a sequence in a denatured nucleic acid to be probed, in relation to its length, to be bound under selected stringency conditions. Primers may vary
15 in length. Preferably such primers should be sufficiently long to hybridize to the modified RNAs in a specific and stable manner. A semi-random primer as the term is used herein, encompasses a class of primers wherein either a discrete portion of the primer is random, while another discrete portion is conserved as well as primers which have nucleotide preferences at particular positions within a sequence. For example, the
20 discrete portion-type primer may have a predetermined adaptor sequence at its 5' end and a random sequence at its 3' end. Alternatively, several preferred primers have nucleotide preferences at specific positions within the primers while other positions are random. A degenerate primer as the term is used herein, encompasses a cocktail or mixture of primers wherein one or more of the possible triplet nucleotide sequences encoding an
25 amino acid is incorporated into the primer sequence. For example, Serine may be encoded by six separate triple sequences (AGU, AGC, UCU, UCC, UCA, and UCG). Thus, a "degenerate" primer may reflect the degeneracy of the nucleotide triplet code. Alternatively, a randomized primer, as the term is used herein, encompasses a primer wherein, the nucleotide at one or more positions may be randomized in order to yield a
30 triplet sequence encoding an alternative or a random amino acid at the position.

An end region as the term is used herein, consists of the end nucleotide and a portion of the region including as much as that half of the entire sequence. For example, the "3' end region" or "3' region" of a primer may include the 3' half of the primer.

A preferred method of hybridization is blot hybridization. See Sambrook et al. 1989 *Molecular Cloning: A Laboratory Manual* 2nd Ed. for additional details regarding blot hybridization. Using this method, separated amplification products are transferred onto a solid matrix, such as a filter. The probe, which is detectable, either directly or indirectly, is hybridized to the solid matrix under appropriate conditions. The matrix is washed to remove excess probe. Thereafter the probe which specifically hybridized to the solid matrix can be detected.

The probe can be DNA or RNA and can be made detectable by any of the many labeling techniques readily available and known to the skilled artisan. Such methods include, but are not limited to, radio-labeling, digoxigenin-labeling, and biotin-labeling. A well-known method of labeling DNA is ^{32}P using DNA polymerase, Klenow enzyme or polynucleotide kinase. In addition, there are known non-radioactive techniques for signal amplification including methods for attaching chemical moieties to pyrimidine and purine rings (Dale, R.N.K. et al, 1973 *Proc. Natl. Acad. Sci. USA* 70:2238-42), methods which allow detection by chemiluminescence (Barton, S.K. et al, 1992 *J. Am. Chem. Soc.* 114:8736-40) and methods utilizing biotinylated nucleic acid probes (Johnson, T.K. et al, 1983 *Anal. Biochem.* 133:125-131; Erickson, P.F. et al, 1982 *J. Immunol. Methods* 51:241-49; Matthaei, F.S. et al, 1986 *Anal. Biochem.* 157:123-28) and methods which allow detection by fluorescence using commercially available products. Non-radioactive labeling kits are also commercially available.

A basic description of nucleic acid amplification is described in Mullis, U.S. Patent No. 4,683,202, which is incorporated herein by reference. The amplification reaction uses a template nucleic acid contained in a sample, two primer sequences and inducing agents. The extension product of one primer when hybridized to the second primer becomes a template for the production of a complementary extension product and vice versa, and the process is repeated as often as is necessary to produce a detectable amount of the sequence.

The inducing agent may be any compound or system which will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, *E.coli* DNA polymerase I, thermostable *Taq* DNA polymerase, Klenow fragment of *E.coli* DNA polymerase I, T4 DNA polymerase, other available DNA polymerases, reverse transcriptase and other enzymes which will facilitate combination of the nucleotides in the proper manner to form

amplification products. The oligonucleotide primers can be synthesized by automated instruments sold by a variety of manufacturers or can be commercially prepared based upon the nucleic acid sequence of this invention.

The degree of hybridization depends on the degree of complementarity, the length
5 of the nucleic acid molecules being hybridized, and the stringency of the conditions in a reaction mixture. Stringency conditions are affected by a variety of factors including, but not limited to temperature, salt concentration, concentration of the nucleic acids, length of the nucleic acids, sequence of the nucleic acids and viscosity of the reaction mixture. More stringent conditions require greater complementarity between the nucleic acids in
10 order to achieve effective hybridization.

"Hybridization" and "binding" in the context of probes, primers and denatured DNA are used interchangeably. Probes which are hybridized or bound to denatured DNA are aggregated to complementary sequences in the polynucleotide. Whether or not a particular probe remains aggregated with the polynucleotide depends on the degree of
15 complementarity, the length of the probe, and the stringency of the binding conditions. The higher the stringency, the higher must the degree of complementarity, and/or the longer the probe.

"Probe" refers to an oligonucleotide designed to be sufficiently complementary to a sequence in a denatured nucleic acid to be probed, in relation to its length, to be bound
20 under selected stringency conditions.

Primers may vary in length. Preferably such primers should be sufficiently long to hybridize to the modified RNAs in a specific and stable manner.

A semi-random primer as the term is used herein, encompasses a class of primers wherein either a discrete portion of the primer is random, while another discrete portion is
25 conserved as well as primers which have nucleotide preferences at particular positions within a sequence. For example, the discrete portion-type primer may have a predetermined adaptor sequence at its 5' end and a random sequence at its 3' end. Alternatively, several preferred primers have nucleotide preferences at specific positions within the primers while other positions are random.

30 A degenerate primer as the term is used herein, encompasses a cocktail or mixture of primers wherein one or more of the possible triplet nucleotide sequences encoding an amino acid is incorporated into the primer sequence. For example, Serine may be encoded by six separate triple sequences (AGU, AGC, UCU, UCC, UCA, and UCG).

Thus, a "degenerate" primer may reflect the degeneracy of the nucleotide triplet code. Alternatively, a randomized primer, as the term is used herein, encompasses a primer wherein, the nucleotide at one or more positions may be randomized in order to yield a triplet sequence encoding an alternative or a random amino acid at the position.

5 Solid matrices are available to the skilled artisan. Solid phases useful to serve as a matrix for the present invention include but are not limited to polystyrene, polyethylene, polypropylene, polycarbonate, or any solid plastic material in the shape of test tubes, beads, microparticles, dip-sticks, plates or the like. Additionally matrices include, but are not limited to membranes, 96-well microtiter plates, test tubes and Eppendorf tubes.
10 Solid phases also include glass beads, glass test tubes and any other appropriate shape made of glass. A functionalized solid phase such as plastic or glass which has been modified so that the surface carries carboxyl, amino, hydrazide, or aldehyde groups can also be used. In general such matrices comprise any surface wherein a ligand-binding agent can be attached or a surface which itself provides a ligand attachment site.

15 As used herein, "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. A pharmaceutically acceptable carrier encompasses any of the standard pharmaceutically accepted carriers, such as phosphate buffered saline
20 solution, water emulsions such as an oil/water emulsion or a triglyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. The invention
25 also provides for pharmaceutical compositions together with suitable diluents, preservatives, solubilizers, emulsifiers and adjuvants. Other embodiments of the compositions of the invention incorporate particulate forms, protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including but not limited to intravenous, intramuscular, parenteral, pulmonary, nasal
30 and oral.

As used herein, an "effective amount" is the amount required to achieve a clinically significant effect. For example a significant reduction of infection, or

reduction of cell growth or reduction of tumor size is a reduction of preferably of at least 30 percent, more preferably of at least 50 percent, most preferably of at least 90 percent. Accordingly, the effective amount will vary with the subject being treated, as well as the condition to be treated. For the purposes of this invention, the methods of administration are to include, but are not limited to administration cutaneously, subcutaneously, intravenously, parenterally, orally, topically, or by aerosol.

The present invention further contemplates therapeutic compositions useful in practicing the therapeutic methods of this invention. A subject therapeutic composition includes, in admixture, a pharmaceutically acceptable excipient (carrier) and one or more of a polypeptide analog or fragment of the provided peptide or peptide composition, a peptidomimetic composition thereof as described herein as an active ingredient. A cocktail of the provided pharmaceutical composition in various combinations is also contemplated.

The preparation of therapeutic compositions which contain polypeptides, analogs or active fragments as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

A polypeptide, analog or active fragment can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium,

calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The therapeutic polypeptide-, analog- or active fragment-containing compositions are conventionally administered intravenously, as by injection of a unit
5 dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

10 The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and
15 are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at
20 one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

As used herein, the term "synthetic amino acid" means an amino acid which is chemically synthesized and is not one of the 20 amino acids naturally occurring in nature.

25 As used herein, the term "biosynthetic amino acid" means an amino acid found in nature other than the 20 amino acids commonly described and understood in the art as "natural amino acids."

As used herein, amino acid residues are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino
30 acid residue, as long as the desired functional property is retained by the polypeptide. NH₂ refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a

polypeptide. Abbreviations for amino acid residues are used in keeping with standard polypeptide nomenclature delineated in *J. Biol. Chem.*, 243:3552-59 (1969).

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues.

Amino acids with nonpolar R groups include: Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Tryptophan and Methionine. Amino acids with uncharged polar R groups include: Glycine, Serine, Threonine, Cysteine, Tyrosine, Asparagine and Glutamine. Amino acids with charged polar R groups (negatively charged at Ph 6.0) include: Aspartic acid and Glutamic acid. Basic amino acids (positively charged at pH 6.0) include: Lysine, Arginine and Histidine (at pH 6.0). Amino acids with phenyl groups include: Phenylalanine, Tryptophan and Tyrosine. Particularly preferred substitutions are: Lys for Arg and vice versa such that a positive charge may be maintained; Glu for Asp and vice versa such that a negative charge may be maintained; Ser for Thr such that a free -OH can be maintained; and Gln for Asn such that a free NH₂ can be maintained. Amino acids can be in the "D" or "L" configuration. Use of peptidomimetics may involve the incorporation of a non-amino acid residue with non-amide linkages at a given position.

Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (i.e., His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces -turns in the protein's structure.

As used herein, "pM" means picomolar, "nM" means nanmolar, "uM, means micromolar, "mM" means millimolar, "ul" or "μl" mean microliter, "ml" means milliliter, "l" means liter.

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The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however,

as limiting the broad scope of the invention. While the invention is described and illustrated herein by references to various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material combinations of material, and procedures selected for that purpose. Numerous variations of such details
5 can be implied as will be appreciated by those skilled in the art.

EXAMPLES

EXAMPLE 1: Molecules specifically expressed in murine stem/progenitor 10 cells

Protein tyrosine kinases and phosphatases expressed in murine hematopoietic stem cells have been previously identified (Matthews, W., et al. 1991; Matthews, W., et al. 1991; Dosil, M., et al. 1996). These molecules play important roles in hematopoiesis
15 and development (Dosil, M., et al. 1996; Mackarechtschian, K., et al. 1995; Shalaby, F., et al. 1995; Kabrun, N., et al. 1997). The present invention contemplates an even more global approach in order to identify molecules specifically expressed in the murine stem/progenitor cell hierarchy. For most of these studies purified (AA4.1+Lin-
/loSca+ckit+) fetal liver cells were used. This population is approximately 1,000-fold
20 enriched for in vivo repopulating activity (LTRA) measured by competitive repopulation (Ly5.1/Ly5.2 congenic system) (Moore, K.A., et al. 1997). All myeloid and lymphoid lineages in primary and in secondary recipients are repopulated by these cells. This degree of enrichment is comparable to the current "state of the art." Other primitive members of the stem/progenitor cell hierarchy share this same cell surface phenotype.
25 These include: (1) LTCIC or cobblestone area forming cells (Ploemacher, R.E., et al. 1989; Ploemacher, R.E., et al. 1991), (2) CFU-blast progenitors, (3) HPP-CFC progenitors (Lowry, P.A., et al. 1995) and (4) stromal-dependent B-lymphoid progenitors (Whitlock, C.A., and Müller-Sieburg, C.E. 1990). The AA4.1+Lin-
/loSca-ckit+ subset is depleted of LTRA but contains significant in vitro progenitor activity. In contrast, no
30 stem/progenitor cell activity is found in the AA4.1 subset (Jordan, C.T., et al. 1990). Short-term (5-7 days) cytokine cultures of stem cells were used to generate committed progenitor populations at the expense of LTRA (Traycoff, C.M., et al. 1996; Peters, S.O., et al. 1995; Knobel, K.M., et al. 1994; Yonemura, Y., et al. 1996). In summary, several

cell populations were defined which represent the beginning, the middle and the end points of the hematopoietic hierarchy. This sets the stage for a comparative analysis of gene expression patterns. A goal of the present invention is to complement the physical and functional phenotypes of stem/progenitor cells with profiles of uniquely expressed genes. It was hypothesized that some of these gene products contribute to the unique biological properties of primitive stem/progenitor cells and therefore are regulators of self-renewal, proliferation, commitment and other processes.

There are a number of ways to compare gene expression profiles which are available to one of skill in the art. It is possible to do this by exhaustive sequencing of representative cDNA collections obtained from stem cell and mature cell sources followed by "electronic subtraction". This approach has several drawbacks. Most importantly, the number of sequences which must be obtained is prohibitive. For a homogeneously pure population this number is on the order of 50,000 (based on approximately 10-20,000 expressed genes in an average cell type and a statistical calculation). In practice, even the most purified stem cell population is heterogeneous. Stem cell enrichment values are only meaningful in relation to an unenriched standard and cannot be converted into an absolute stem cell number. It has been documented that cell populations with the same cell-surface phenotypes can differ in biological activity. The unique properties of stem cells also suggest caution when extrapolating from expressed gene numbers in other cells. In short, the extent of sequencing necessary to ensure complete coverage of gene expression in stem cells is not possible to estimate. Normalization procedures (Uchida, N., et al. 1995; Patanjali, S.R., et al. 1991; Soares, M.B., et al. 1994) designed to "equalize" the mRNA abundance classes are not advisable because they obliterate potentially important quantitative expression differences. Additionally, a high-throughput sequencing effort is not applicable to numerous libraries. Comparisons of gene expression in diverse sources of stem cells will provide valuable information. An elegant technique, Serial Analysis of Gene Expression (SAGE) permits the rapid acquisition of thousands of DNA sequence "tags" (Zhang, L., et al. 1997). This technology was not considered herein because the size of each sequence "tag" is very small (10 base pair, bp). Therefore, SAGE is only informative in two extremes; exact nucleotide matches or no matches to sequences in the databases. This limits database comparison to the same species from which the "tags" originate. A key component of the strategy presented herein relies on broad bioinformatic database comparisons. In

addition, even with a specific "tag" one still needs to obtain a full-length cDNA clone for functional studies. Analysis of gene expression can be done in single progenitor cells after these form a colony "start" (Brady, G., et al. 1990; Brady, G., and Iscove, N.N. 1993). Replating of the sibling cells in a "start" colony allows the approximation of the
5 lineage potential present in the starting cell. This technique suffers from several drawbacks. First, it is limited to 3', non-coding ends, thus preventing protein database comparisons. Second, the technique relies on cell growth, thus it is not suitable for analysis of quiescent cells. Moreover, approaches to allow colony-formation by true stem cells with a retention of primitive properties are in their infancy (Ball, T.C., et al. 1995;
10 Trevisan, M., et al. 1996). Third, the technique does not take into account stochastic models of stem cell behavior (Ogawa, M. 1993). To accurately reveal physical and functional properties of stem cells it is wise to analyze populations where stochastic differences would average out. Single-cell derived cDNA populations could however, provide valuable "adjunct" material for more refined gene expression screens. There are
15 many techniques to physically identify differentially expressed genes. For these and other reasons, most notably the technical and economic ease with which physical pre-enrichments of cDNA libraries can be achieved, the studies described herein began with cDNA libraries which are highly enriched in differentially expressed sequences. The integration of individual techniques served to overcome the inherent limitations of each
20 technique. Three strategies were successfully employed: (1) Differential Display (DD) (Liang, P., et al. 1994; Bauer, D., et al. 1993), (2) Representational Difference Analysis (RDA) (Braun, B.S., et al. 1995; Hubank, M., and Schatz, D.G. 1994; Diatchenko, L., et al. 1996) and (3) standard subtractive hybridization (Li, W.-B., et al. 1994; Harrison, S.M., et al. 1995). The latter underlies the present invention. A key feature is that the
25 differentially expressed cDNAs have a high probability of being full-length. This facilitates a rapid transition to functional studies. The two former techniques were utilized because of "visual" nature (DD) and the ability to generate representative, differentially expressed probe populations (RDA) in a rapid manner.

Murine stem cell gene expression profiles. As a first step, a series of high quality,
30 representative cDNA libraries were generated. The cDNA populations were directionally cloned into the pSport-1 or pSport-2 plasmids (BRL-Gibco). The most important libraries originate from purified stem cells. In one case, enough AA4.1+Lin-
/loSca+ckit+cells were purified to allow construction of a non-based library using

standard methodologies. This library contains $\sim 4 \times 10^6$ independent recombinants (average cDNA insert size of 1-2 kilobases, kb). A second library was constructed using a new PCR-based technology called cap-finder (Clontech) designed to yield full-length cDNA copies. cDNAs ranging from 1 to 4 kb were commonly attained using this technique. For cap-finder procedures the purified cells were processed into DNase-I digested, poly-A⁺ mRNA according to microscale procedures routinely in use. The synthesis of cDNA was done with an aliquot of mRNA corresponding to approximately 20,000 cell equivalents. It has not been necessary to use less material, therefore this is not a lower limit. A Not I restriction site was included in the 3' reverse transcriptase primer (cap-finder, version 2) to facilitate directional cloning. An aliquot of the cDNA was amplified for varying PCR cycle numbers, and analyzed by Southern blots (pseudo-Northerns) for the presence of full-length copies of b-actin, GAPDH, CD18, flk2/flt3, cdk4, CD34 and other mRNAs. Optimal cycle numbers were used to amplify the remaining cDNA. The cDNA was cloned into the pSport-1 plasmid. The AA4.1+Lin-
15 /loSca+ckit+ cap-finder library contains $\sim 3 \times 10^6$ independent recombinants. Single-pass sequence analysis of random clones from this library indicated that 95% were full-length (based on sequences with an exact match in Genbank). Much more extensive sequencing of numerous clones from subtracted libraries has confirmed this. Other libraries constructed in similar ways include two libraries from AA4.1 cells, and two libraries from
20 AA4.1+Lin-/loSca-ckit+ cells. Finally, a library was constructed from AA4.1+Lin-/loSca+ckit+ cells cultured for 5-7 days in a differentiation promoting cytokine cocktail (IL3, IL6, KL). Competitive repopulation and in vitro progenitor assays confirmed a complete loss of LTRA with a significant retention of progenitor cells. All of the cDNA libraries are large ($> 2 \times 10^6$ independent recombinants) and of high quality (1-2 kb
25 average insert size). In summary, the most primitive, intermediate and most mature members of the hematopoietic hierarchy were "converted" into representative panels of expressed genes.

Subtractive Hybridization. These cDNA libraries were used in subtractive hybridizations to enrich for differentially expressed genes. Target libraries from
30 AA4.1+Lin-/loSca+ckit+ cells were subtracted with an AA4.1 driver cDNA library. This yields a population of cDNA clones which is enriched in sequences expressed in primitive stem/progenitor cells but not in mature cell types. In practice, a single-stranded target library is hybridized to an excess of in vitro synthesized biotinylated RNA from the

driver library as described . The opposite orientations of the cloning sites in pSport-1 and 2 insure target and driver complementarity. The target library was enzymatically converted to a single-stranded form using gene II protein and exonuclease III (Gene-Trapper protocols, BRL-Gibco). Previously, this was done by infection with M13 helper
5 phage often resulting in a bias for small cDNA inserts. Here, driver/target combinations were subtracted two times in order to facilitate the removal of commonly expressed sequences (verified by elimination of "housekeeping" genes such as b-actin and GAPDH). Concomitant enrichment of known, differentially expressed genes is also verified. Generally flk2/flt3 and CD34 probes were used. Both are expressed in the
10 AA4.1+Lin-/loSca+ckit+ subpopulation but not in the AA4.1 population. Following subtraction, the relative number of clones is reduced by up to 200-fold. In some cases (subtractions with AA4.1 material) the number of clones which "survive" the subtraction is on the order of 10-20,000. Because individual sequences may be represented more than one time, this does not necessarily imply that there are 10-20,000 differentially
15 expressed genes. The exact number of unique sequences (complexity) in the pool of subtracted clones must be determined. A more thorough discussion of complexity is found in a subsequent section. These subtracted libraries should be enriched for sequences expressed in the primitive portion of the stem/progenitor cell hierarchy; that is in stem cells and/or in primitive clonogenic progenitor cells. Two other subtracted
20 libraries, potentially enriched for sequences expressed in the most primitive stem cell but not in clonogenic progenitors were derived by subtracting the AA4.1+Lin-/loSca+ckit+ libraries with material from the closely related AA4.1+Lin-/loSca-ckit+ subpopulation.

Each subtracted library is arrayed at high density onto nylon membranes. Each clone in the array has a unique address in microtiter plates. A density of 20-30,000 clones
25 on a 22 x 22 cm. membrane is practical. Because of the inherent "noise" in any subtraction scheme, a positive selection criterion may be imposed in order to focus on true differentially expressed sequences. Details are presented in a subsequent section. To analyze the subtracted libraries, a high throughput sequencing effort was employed using three libraries. These are: 1) two AA4.1+Lin-/loSca+ckit+ cell libraries (standard and cap-finder) subtracted extensively with AA4.1 cell material and 2) a standard
30 AA4.1+Lin-/loSca+ckit+ library subtracted with AA4.1+Lin-/loSca-ckit+ material. An average of 400 bp of 5' sequence was obtained from about 1000 clones. To facilitate a rational handling of sequence information and to focus attention on a small number of

clones for functional analysis a "flow of information" strategy was devised. (See, Figure 1).

A major component of this strategy is bioinformatics. This can be global (comparisons with outside databases) and local ("in-house" analyses within an individual library or comparison of several libraries). A relevant example of the latter is comparison of genes from murine stem cells with genes derived from their human counterparts. Global bioinformatic analysis provides much information. First, it establishes if a given sequence corresponds identically or closely to an already identified gene in the mouse, human or other mammalian species. Such homologies can be detected at the nucleotide level. This can provide evidence of hematopoietic expression for a previously described gene. Second, where the homologies are statistically significant but not identical, novel members of gene/protein families can be identified. Third, a wider net can be cast over the databases by using conceptual translation of a sequence in the homology comparisons. The Examples provided herein illustrate the power of this approach in revealing similarities to proteins from invertebrate organisms such as *Drosophila*, *C. elegans*, and even yeast. In many cases these proteins have functions which have been uncovered by the analysis of mutants. Based on protein homologies "virtual links" are drawn between developmental regulation in invertebrates (such as in germ-line development) and in hematopoietic stem cells. The Notch/Notch ligand pathway first defined in invertebrate cell-fate determination and recently implicated in hematopoietic regulation is a good example.

A number of other putative proteins which share homology with key *Drosophila* proteins have been identified. Bioinformatics facilitates the recognition of peptide motifs such as EGF-like repeats, Ig-like domains or Zn-finger modules. Fourth, because the databases are annotated, predicted protein sequences can be assigned to cellular processes such as signal transduction pathways or apoptosis. It is also possible to categorize clones according to potential involvement in other mammalian stem cell systems such as the intestine. Fifth, it is feasible to perform virtual expression studies and to construct overlapping EST contigs which can yield virtual full-length cDNAs.

The following discussion summarizes the general murine findings and highlights a panel of "interesting" subtracted clones. A number of full-length sequences have been determined. Bioinformatic analysis summaries on a collection of 863 clones derived

from AA4.1+Lin-/loSca+ckit+ libraries subtracted with AA4.1 RNA are shown in Table 1.

The present invention encompasses numerous bioinformatic search and comparison parameters. This is the first analysis of its kind in the hematopoietic system and several important points emerge. First there is a high proportion (~50%) of novel sequences. Sixteen percent do not show homologies in any EST database queried. A preliminary analysis of a small sequence set from an AA4.1+Lin-/loSca+ckit+ library depleted of sequences in common with the AA4.1+Lin-/loSca-ckit+subset has indicated an even higher percentage of novel genes. These are higher percentages than would emerge from a random analysis of an unsubtracted library. This was directly addressed by performing these analyses on a similar number of sequences selected at random from one of the mouse EST databases. Second, among the ~50% of the clones which are significantly homologous to previously identified genes or proteins, the proportion of homologies to "housekeeping" genes is low. This underscores the effectiveness of the subtraction strategy. Third, an internal analysis of the data base has revealed few redundancies. This illustrates the degree of gene expression diversity between the two endpoints of the mouse hematopoietic hierarchy; thus supporting the starting hypothesis. A recent report has suggested that many sequences in mature blood cells are expressed in primitive, non-committed cells (Hu, M., et al. 1997). The data indicate that many of these are removed by the subtraction; thus uncovering a large, previously not described set of genes. Fourth the sequences with homologies to known genes or proteins can be subdivided according to protein families and putative function. Interestingly, a large percentage (32%) fall into the signaling protein category. Examples of these are described herein below.

As part of a more sophisticated bioinformatics approach, automation of database searches, information cross-referencing, and annotation was employed. An illustrative example is automated weekly database queries with the sequence set. New, previously-unidentified homologies were automatically noted and reported. In the mouse studies several genes were encountered whose expression would be predicted to differ between the AA4.1+Lin-/loSca+ckit+ and the AA4.1- populations. Both murine CD34 and flk2/flt3 were identified in the sequenced population. This provides a good internal control for the screening strategy. As shown in Table 2, a "short list" of identified

molecules was generated based on bioinformatics and in many cases, expression-specificity verification. The features of some of the molecules merit discussion.

The SA49P1 clone is homologous to sex comb on midleg; a member of the *Drosophila* Polycomb group of zinc-finger transcriptional repressors (Bornemann, D., et al. 1996). Polycomb proteins are regulators of homeobox (HOX) genes and maintain the developmental stability of transcriptional states (Simon, J. 1995). There is currently great interest in HOX gene function in the biology of hematopoietic stem cells (Sauvageau, G., et al. 1995; Lawrence, H.J., et al. 1996). The C4-80 gene is homologous to the *Drosophila* cornichon gene (Roth, S., et al. 1995). Cornichon is required during oogenesis for the induction of follicle cells, which provide the environment that supports oocyte development. Cornichon is a component of the *Drosophila* EGFR signaling pathway (Neuman-Silverberg, F.S., and T. Schupback 1996). The LL2-12 gene is similar to the *Drosophila* neurogenesis gene brainiac. Mutants defective in this extracellular gene product show neural hyperplasia. Brainiac also plays a role in oogenesis (Goode, S., et al. 1992; Goode, S., et al. 1996). Mosaic experiments show that brainiac is required in the developing oocyte. Brainiac involvement in the *Drosophila* EGFR pathway has been suggested. The LL2-35 gene is similar to the *Drosophila* germ cell-less gene (Jongens, T.A., et al. 1992). The product of this gene is required for the specification of the *Drosophila* germ line. The ectopic expression of germ cell-less causes somatic cells to adopt the characteristics of pole cells (destined for the germ line). The homologies of the above clones to *Drosophila* genes were identified at the predicted amino acid level. This underscores the utility of the bioinformatic approach. In all four cases the cDNAs represent novel unpublished murine genes. Clearly, the involvement of three of the *Drosophila* genes in the ultimate stem cell system, the germline, coupled with the identification of homologous genes expressed in mouse hematopoietic stem /progenitor cells gives food for thought. Four other cDNAs identified are homologous to the *Drosophila* genes dishevelled and smoothened (mouse homologs are already described) as well as to kelch and discs large (not previously described in the mouse). DD116 was originally isolated in a DD comparison of AA4.1+ vs. AA4.1 cells. A cDNA clone identified with the DD116 probe was sequenced; the predicted protein is homologous but not identical to the murine beige gene product. The beige mutation in the mouse causes bleeding, immune system disorders and a coat-color phenotype (Perou, C.M., et al. 1996; Fukai, K., et al. 1996; Barbosa, M.D., et al. 1996). Murine beige is thought to be the

homologue of a gene responsible for the Chediak-Higashi syndrome in human. This novel gene is likely to be the second member in the beige family; it is designated herein as "taupe". Two of the cDNAs in Table 2 were identified in a DD comparison of AA4.1+Lin-/loSca+ckit+ cells and cytokine differentiated, cultured cells. One focus was molecules whose expression disappeared with the loss of stem cell activity in culture. Cyt28 and Cyt19 fulfilled this criterion and were used to isolate full length clones which have been completely sequenced. Cyt28 encodes a novel, seven-transmembrane domain receptor in the same family as mouse F4/80 and human CD97 (secretin receptor superfamily) (Hamann, J., et al. 1995; Baud, W., et al. 1995). Cyt 19 is a novel putative methyltransferase. In both of these cases protein family assignments were made after extensive bioinformatic analyses. At least in the case of Cyt28, an antibody will be a useful reagent because it may provide a means to further subdivide the stem/progenitor cell population. Such antibodies are provided by the present invention. The cDNA SA61 is similar to a newly discovered molecule called p62dok. p62dok is a tyrosine phosphorylated protein which binds to rasGAP and is likely to be a common target for numerous tyrosine kinases including Abl and ckit (Carpino, N., et al. 1997; Yamanashi, Y., 1997). Interestingly, p62dok is also constitutively phosphorylated in hematopoietic progenitor cells from chronic phase CML patients (Carpino, N., et al. 1997). The predicted protein encoded by SA61 appears to be a second member of this protein class. Other representative molecules are listed in Table 2. Two cDNAs were identified as homologous to putative apoptosis regulators (SBSA56 and LL5-68, two cDNAs homologous to genes translocated in leukemias (LL5-03 and B2-67), several homologies to putative chromatin proteins (C4-23, C3-25 and LL2-89) and a LIM-domain encoding cDNA (LL5-96). Most interestingly, three cDNAs (B3-77, C2-48, and LL2-76) are homologous to genes expressed in intestinal crypts or during intestinal development.

Isolated cDNAs were hybridized to slot blots representing globally amplified cDNAs from pools of individual progenitor and mature cells. Because the developmental potentials of these cells have been measured (by replating of siblings) it is possible to graphically represent the expression of a gene in various stages of the hierarchy. Three examples are shown in Figures 2A-2C. The Smc-34 cDNA is a completely novel sequence with a predicted leucine zipper and several potential membrane spanning domains. These examples underscore the value of "interfacing" the above-described approach with a single cell approach. Thus, it was demonstrated that a given gene is

expressed in at least multipotential progenitor cells and other members of the progenitor cell hierarchy.

Human stem cell gene expression profiles. It is also a goal of the present invention to identify genes expressed specifically in human stem cells. It is useful to use
5 a multi-species (mouse and human) approach to define stem/progenitor cell-specific gene profiles. One goal is to integrate mouse and human information. However, rather than proceeding directly to human homologs of the mouse genes already identified, an independent human effort was initiated. The rationale for this is multi-faceted. First, it is possible that some aspects of human and mouse stem cell biology are regulated in
10 different ways. Although it is likely that most regulatory pathways will be conserved, it must be kept in mind that many properties ascribed to the most primitive stem cell population have been rigorously proven only in the mouse. Clearly a human lymphoid-myeloid stem/progenitor cell exists. However, the exact degree of proliferative capacity (the ability to give rise to oligoclonal hematopoiesis) as well as the exact spectrum of
15 differentiation potentials of human stem cells have not yet been accurately measured. Several potential differences between mouse and human have already been mentioned. One additional indicator of differences may be the difficulty of gene-transfer into stem cells from large animals (Larochelle, A., et al. 1997). This is not likely to result solely from the quiescent status of the most primitive cells. Second, while it is usually possible
20 to find human homologs for individual mouse genes by manipulating hybridization stringency, such conditions will vary for different genes. Therefore, to find human homologs for a large pool of mouse genes (i.e. 100) may be more labor intensive and costly than to independently determine a sequence profile of differentially expressed genes from human stem/progenitor cells. Relationships to the murine panel can then be
25 determined electronically where it is easy to manipulate comparative parameters. Clearly, for some individual mouse genes it will be of great importance to physically identify human homologs. In some cases it may be possible to use existing human EST databases to quickly obtain the sequence of a human homolog. Third, the availability of a large panel of human sequences specifically expressed in stem/progenitor cells lends
30 itself to the application of various chip and array technologies. Such technologies will be instrumental in identifying which subsets of human stem cell specific genes are up or down-regulated in the highly clonogenic stem cells from diseases such as Acute Myelogenous Leukemia (AML) and other leukemias.- Highly purified human stem

cells for cDNA libraries were obtained from normal donor BM. CD34⁺ cells were obtained from the mononuclear fraction using an immuno-magnetic affinity device (Miltenyi MACS column) and stained with a mixture of monoclonal antibodies (mAbs) designed to identify both primitive and mature cell surface markers. To identify antigens on mature cells a lineage (Lin) cocktail of FITC conjugated mAbs was used. These mAbs were directed against: CD3, CD11b, CD15, CD19, CD20, glycophorin A, and CD71 (CD3, CD15, CD19 and CD20 from Becton Dickinson, CD11b and glycophorin A from Coulter, and CD71 from Ortho Diagnostics). To identify primitive cells mAbs were used which were directed against CD34 (biotin conjugated and detected via a streptavidin-allophycocyanin reagent; Coulter) and either CD38 (phycoerythrin conjugated, Becton Dickinson) or CD90 (Thy1) (phycoerythrin conjugated; Pharmingen). The stained cells were analyzed using a dual laser Becton Dickinson FACStar Plus flow cytometer. Cells of the desired phenotype were sorted into siliconized tubes. To obtain material from cultured cells, CD34⁺ enriched populations (isolated as described above), were first cultured in serum-free medium (IMDM, 5 mg/ml low density lipoprotein, 2 mM glutamine, human serum albumin, insulin, and transferrin,) supplemented with IL-3 (5ng/ml), KL (25ng/ml), and FL (25ng/ml). After 1-4 days of culture, the cells were purified as described above. Human libraries were constructed using the cap-finder technology, version 2. Amplified cDNAs originating from several independent BM donors were pooled. Representative cDNA libraries have been constructed from the following sources: (1) BM CD34⁺Lin⁻ cells (7.1X10⁵ independent recombinants), (2) BM CD34⁺Lin⁻CD38⁺ cells (1.9X10⁶ independent recombinants), (3) BM CD34⁻ cells (1.6X10⁶ independent recombinants), (4) CB CD34⁺ cells (4.3X10⁵ independent recombinants), and (5) CB CD34⁻ cells (2.9X10⁵ independent recombinants). The average cDNA insert size in all libraries is 1-2 kb. According to the comparative biological properties of the source material used for the mouse and human cDNA libraries, the following parallels can be drawn: (1) mouse AA4.1⁺Lin⁻/loSca⁺ckit⁺ $\cong$ human CD34⁺Lin⁻, (2) mouse AA4.1⁺Lin⁻/loSca⁻ckit⁺ $\cong$ human CD34⁺Lin⁻CD38⁺ and (3) mouse AA4.1 $\cong$ human CD34⁻. Thus, in both species, collections of clones representing the beginning, the middle and the end-points of the hematopoietic hierarchy have been generated. Using procedures described above, the human libraries were subtracted and arrayed 3,000 clones from two libraries: (1) BM CD34⁺Lin⁻ subtracted

with BM CD34+Lin-CD38+ material (referred to as: 38-)38+) and (2) BM CD34+Lin-
subtracted with BM CD34- material (referred to as: 38-). Before initiating a sequencing
effort pilot studies were performed to improve the resolution of the screen. Specifically,
in order to develop techniques which would eliminate as much non-specific "noise" as
possible from the libraries. RDA (PCR-Select, Clontech) were used . There are several
5 salient features of RDA. First, it is rapid (2-3 days) and applicable to small cell numbers
(ie. several thousand). It does not require the generation of cloned cDNA libraries
therefore, numerous comparisons can be made simultaneously. Second, it yields short
cDNA fragments (generated by 4-cutter restriction enzymes) which are representative of
10 differentially expressed mRNAs. Third, it is based on the suppression PCR technique
which prevents overamplification of abundant messages (Diatchenko, L., et al. 1996) . In
addition, sequences expressed in both populations in an RDA comparison do not amplify
exponentially. Differentially expressed populations of cDNA fragments are not obtained
by physical enrichment but rather by selective PCR amplification. Fourth and most
15 importantly, it simultaneously yields two populations each representing differentially
expressed genes in one of the two starting samples. This bi-directionality is valuable
because as discussed below it can simultaneously reveal up and down regulated genes.
RDA comparisons were made between CD34+Lin- and CD34+Lin-CD38+ and between
CD34+Lin- and CD34- cDNAs. The latter comparison was verified by hybridization. As
20 shown in Figure 3A (right panel), the control, non-subtracted RDA cDNA population (38-
) contains b-actin sequences which are missing in the two subtracted RDA populations,
38- and 38-)38+. A differentially expressed gene (HDD-2, described below) is enriched
in the 38- RDA population and at least retained in the 38-)38+ RDA population (Figure
3A, left panel). Two, bi-directional, RDA cDNA populations (38- and the converse 34-
25)38) were used to probe (See Figure 3) duplicate arrays of a subtracted 38- library (Figure
3B). The correct RDA probe hybridizes to considerably more clones than the incorrect
probe (compare the greater numbers of spots in the left as compared to the right panel).
Hybridization signals for each clone are doublets due to the arraying technique.
Hybridization signals with the incorrect probe (right panel) suggest that further
30 improvements to this strategy are worth pursuing. Because the subtractions are based on
different technologies, it was reasoned that clones which "survived" the library
subtraction and hybridized preferentially to the "correct" RDA probe would be more
likely to represent true differentially expressed genes. Preliminary data from the mouse

studies had already suggested that this was indeed the case. The arrayed clones were also hybridized to probe populations from the entire starting non-subtracted libraries in order to eliminate residual cDNA clones corresponding to abundant mRNA species.

In one analysis, a total of 77 clones (49 from the 38- subtraction and 28 from the -
5 38-)38+ subtraction) which "obeyed" the RDA criteria were sequenced from the 5' end. In order to verify expression specificity, a series of pseudo-Northern blot analyses were performed. cDNA populations from independent purified sources of material was amplified. This is of particular importance with an outbred species such as humans. In these experiments additional material from CD34+Lin-CD90+ (Thy1+) (three separate
10 purifications) and their CD90- (Thy1-) counterpart subpopulations was also included. Cytokine-cultured samples were also included. These were CD34+Lin- cells and their CD38+ counterparts purified after 1, 2 and 4 days of culture. The collective panel of cDNAs also includes four independently purified CD34+Lin- populations and their CD34+Lin-CD38+ counterparts. All of these amplified cDNAs have been arranged on
15 numerous pseudo-Northern blots. In short, this allowed us to evaluate the expression of a given "sequence tag" in stem cell populations isolated by two different criteria, from a number of independent donors and after cytokine culture. Several interesting genes emerged from the sequence data set. Individual clones obtained from the -38-)38+ subtraction are designated 38 letter/number (i.e., 38A1) while those from the 38-)34+ subtraction are designated 34 letter/number (i.e., 34A1). One clone (38B5) was identified
20 and determined to be human flk2/flt3. A second interesting clone (34B4) is closely related to a gene encoding TINUR (Figure 4A). Clone 34B4 may be a novel variant of TINUR due to a 25 amino acid, in-frame deletion. TINUR was identified as an orphan member of the steroid receptor superfamily (NGFI-B/nur77 subfamily)(Okabe, T., et al.
25 1995). TINUR has also been implicated in apoptosis. An additional clone (34F4) is highly homologous to DAP-kinase. This protein is a serine-threonine kinase which has been implicated in cytokine (IFN- -induced apoptosis (Deiss, L., et al. 1995) . Clone 34F4 (DAP-Kinase) and 34B4 (TINUR) both exhibit a stem-cell restricted expression pattern. Clearly, the identification of two genes whose products are implicated in
30 apoptosis and whose expression is largely restricted to human stem cells is of interest. In addition, a cDNA (34A5) was identified which is closely related to the MLF1 gene which is a translocation partner in t(3;5)(q25.1;q34)(Yoneda-Kato, N., et al. 1996) . This translocation is associated with Myelodysplastic Syndrome (a stem cell disease) and

AML. The sequence homologies and restricted expression pattern of 34A5 is shown in Figures 4B and 4C. In Figures 4C and 4D (and also 5A, 5B, and 6B) there are twenty-one samples of capfinder-amplified cDNA from various hematopoietic populations. From left to right these are: four CD34+Lin- populations, three CD34+Lin-CD90+
5 populations, two CD34- populations, four CD34+Lin-CD38+ (obtained from the same BMs as the CD38- samples in lanes 1-4), two CD34+Lin- samples (obtained from the same BMs as the CD90+ samples in lanes 5 and 6), three CD34+Lin- populations obtained after 1, 2 or 4 days of culture and finally their three CD34+Lin-CD38+ counterparts. A recent study shows that, at least with some types of AML, the disease
10 can be transferred into NODSCID mice only by the leukemic CD34+CD38- subpopulation (Bonnet, D., and Dick, J.E. 1997) . These demonstrate MLL1 expression in normal stem cells. As shown in Figure 4D, nucleophosmin (NPM), the partner in this translocation was also identified. This is an example of non-specifically expressed "noise" in the screens. The 38G2 cDNA is closely homologous to the LTG9/MLLT3
15 gene located on 9q22 and involved in t(9;11) leukemia (Iida, S., et al. 1993) (Figure 4E). A degree of stem cell expression specificity has also been observed. All of the above cDNA clones are likely to be identical to the homologous, previously identified genes. However, the suggested involvement of DAP-kinase and TINUR in apoptosis necessitates their inclusion in any comprehensive consideration of stem cell apoptotic pathways.
20 Similarly, the expression of two genes associated with myeloid leukemias bears on any speculation regarding the primary transformation target cell as well as the origins of the ultimate clinical phenotype in these and other leukemic disorders.

Other known genes to were found that exhibit identity or very close homology including: (1) G0S3, a fos-related gene (Heximer, S., et al. 1996) (Figure 5A) and (2)
25 HLA-DR (Figure 5B). G0S3 shows a specific expression pattern, while HLA-DR expression appears to be more variable. The expression status of Class II MHC on the most primitive human BM stem cell population is not entirely clear. It has been suggested to be present in a primitive, multipotent progenitor population but not in the most primitive stem cells (Sutherland, H.S., et al. 1989; Verfaillie, C., et al. 1990) . If
30 true, this may suggest an additional negative selection parameter for future experiments designed to subdivide the stem/progenitor cell hierarchy. Of the first 75 sequences, 22 have no homologies in the databases or homologies only to ESTs. Expression analyses on these clones are in progress. One gene of particular interest is called HDD-2 as well as

34B5, 34E1 and 38A11. The three latter designations reflect its isolation from both the 38- as well as the 38-)38+ subtracted libraries. The designation HDD-2 reflects its independent isolation in a limited DD "first look" at molecular differences in the purified cell populations. The likely full-length sequence of HDD-2 is ~500 bp. It contains a short open reading frame of 89 amino acids (SEQ.ID.No.: 71). The predicted peptide sequence is shown in Figure 6A. The 3' cDNA sequence contains a poly-A tail preceded by the canonical AATAAA poly-adenylation signal. Neither the nucleotide nor the predicted protein sequences of HDD-2 show homologies in any known gene or EST database. The expression profile of HDD-2 shown in Figure 6B, demonstrates that it is stem cell restricted. Also shown below (Figure 6C) is HDD-2 hybridization to a dot blot with numerous human pA+ mRNA samples (Clontech). HDD-2 hybridization is only visible in kidney (the other "spots" are background). It was confirmed that HDD-2 corresponds to a single-copy human gene by genomic Southern blot (Figure 6D). In summary, the results of this very low throughput human sequencing effort substantiate the overall approach; that judicious pre-enrichments and selections will result in rapid identification of biologically interesting and often novel genes. Most importantly, these studies firmly establish the existence of genes whose expression correlates with the most primitive stem cell phenotype.--

Other data were generated using (1) a murine stromal cell line to support enriched human stem/progenitor cells, and (2) tetracycline regulatable retroviral expression vectors. The stromal cell line AFT024 is efficient in long-term, in vitro maintenance of LTRA in purified murine fetal liver and adult bone marrow populations (Terstappen, L.W.M.M., et al. 1991) . Additional data demonstrated highly-efficient retroviral-mediated gene-transfer into murine LTRA as well as into primitive in vitro progenitors during the AFT024 cocultures. Further, it has been shown that AFT024 is very effective in supporting ELTCIC. Specifically, the CD34+CD38- immunophenotype as well as the functional capacity of these cells is maintained. The latter was measured in limiting-dilution replating experiments. Moreover, it has been shown that limiting numbers of human CD34+Lin- cells can give rise to both B cells and NK-cells when cultured on AFT024. However, the supportive activities of AFT024 on mouse and human stem/progenitor cells have been indistinguishable. In addition, more than 500 sequences have been analyzed from an AFT024-specific subtracted cDNA library. A number of candidate stem cell regulators have been identified. Three of these are dlk (Moore, K.A.,

et al. 1997), a novel BMP/TGF- β superfamily member and a novel selectin-related molecule. The present invention contemplates identifying human stem/progenitor cell receptors and/or ligands for the AFT024 specific proteins.

In order to facilitate functional studies of stem cell gene products several retroviral gene-transfer vector systems were constructed and characterized. All of these employ the 293T cell retroviral packaging system (Kinsella, T., and Nolan, G. 1996). High titers of virus can be produced transiently without the time and labor consuming effort required for stable producer cell lines. Large cDNA populations can also be converted into virus populations (Kitamura, T., et al. 1995). It is preferable to have inducible (or repressible) vectors which are also selectable. Also, the single-transcription unit tetracycline (tet) repressible vector was modified (Hofmann, A., et al. 1996). This vector includes an enhancer/promoter deletion in the 3' LTR. The tet system is currently one of the best inducible expression systems available; regulation over a several hundred-fold range of expression is observed (Shokett, P.E., and Schatz, D.G. 1996). Cloning sites were introduced in order to insert cDNAs in a sense or antisense orientation. A green fluorescent protein (GFP) marker was also included in these vectors driven by a thymidine-kinase promoter (TK-GFP) (Yang, T., et al. 1996; Cheng, L., and Kain, S. 1996). The cDNA fragment of interest is under tet regulation while GFP is constitutively expressed in transduced cells. In order to confirm this, NIH3T3 cells are infected with a LacZ virus and GFP+ cells sorted. The GFP+ cells express LacZ in the absence of tet, while LacZ expression is undetectable in most cells after the addition of tet. The titer of this model construct is approximately 10^4 /ml. which is suitable for tissue culture studies.

Table 1. Known genes (or extensive aa homology): 436 (50.5%)

Grouped by function	
by nucleotide: 407	Signaling/receptors: 133
by amino acid only: 29	Translational/post-: 62
	Structural: 49
to mouse: 230	Transcriptional/post-: 45
to human/other: 206	Cell fate: 35
	Other: 87
	Unknown function: 25

Novel genes: 427 (49.5%)

Homologous only to expressed sequence tags: 288 (33.3%)

No homology to any known nt or aa sequences: 139 (16.2%)

Table 2.

Putative family	Clone	Method	Cells compared	Notes
7-transmembrane receptor	Cyt-28	diff. display	SC* fresh vs. 7d-cultured	secretin R superfamily
Methyltransferase	Cyt-19	diff. display	SC fresh vs. 7d-cultured	
	C3-54	subtraction	SC vs. AA4'	
Aspartyl protease	SA7	subtraction	SC vs. AA4'	
Signal transduction molecules	SA61	subtraction	SC vs. AA4'	dok family member
	LL2-02	subtraction	SC vs. AA4'	BTK associated
beige-related proteins	DD116	diff. display	AA4' vs. AA4'	contains WD repeat
Transcriptional regulators	SA49P1	subtraction	SC vs. AA4'	polycomb homol.
	LL5-96	subtraction	SC vs. AA4'	includes LIM domain
G-protein signaling	LL4-39	subtraction	SC vs. AA4'	cell cycle transition
	B1-66	subtraction	SC vs. AA4'	similar to BL34
Apoptosis-related genes	SBSA56	subtraction	SC vs. AA4'	SMT3A-related
	LL5-68	subtraction	SC vs. AA4'	sim. to Requiem transcript. factor
Chromatin proteins	C4-23	subtraction	SC vs. AA4'	yeast HST2-like
	C3-25	subtraction	SC vs. AA4'	NHP2-like
	LL2-89	subtraction	SC vs. AA4'	yeast SIS2-like
Other stem cells (intestinal crypts)	B3-77	subtraction	SC vs. AA4'	homologous to A4 gene
	C2-48	subtraction	SC vs. AA4'	homologous to C101
	LL2-76	subtraction	SC vs. AA4'	homologous to EDPF
Genes involved in leukemogenic translocations	LL5-03	subtraction	SC vs. AA4'	t(1:1)(q21;q23)
	B2-67	subtraction	SC vs. AA4'	t(X:14)(q28;q11)
Homologues of Drosophila developmental genes	LL2-35	subtraction	SC vs. AA4'	germ cell-less
	LL2-12	subtraction	SC vs. AA4'	brainiac
	C4-80	subtraction	SC vs. AA4'	cornichon
	B4-14	subtraction	SC vs. AA4'	discs-large

*The designation "SC" refers to sorted day 14 fetal liver cells having the phenotype AA4.1⁺Lin⁻Sca⁺ckit⁻.

EXAMPLE 2: Molecular cloning and characterization of AA4, an early marker of hematopoietic development

In this example, the expression cloning and molecular characterization of AA4, a surface marker expressed on hematopoietic stem and progenitor cells is described. The results demonstrate that AA4 is a 130kDa type I glycosylated membrane protein whose structural organization suggests a role in cell adhesion. Expression analysis showed that high levels of AA4 are found in lung, heart, and bone marrow. It is not found in undifferentiated ES cells, but its expression is upregulated as these cells differentiate into colonies of hematopoietic precursors and endothelial cells. In the hematopoietic system, expression of AA4 correlates with the expression of stem cell markers CD34 and CD43. Functional studies revealed that AA4 coimmunoprecipitates with CD34 and CD43, suggesting that these proteins form a macromolecular complex which functions in the regulation of cell adhesion, proliferation and/or differentiation of hematopoietic cells.

Introduction

Monoclonal antibody AA4.1 was first described more than a decade ago (McKearn et al., 1984) and since then it has become a useful marker for the isolation and analysis of hematopoietic cells (McKearn et al., 1985; Jordan et al., 1990; Fujimoto et al., 1996). A number of works have shown that the antigen recognized by AA4.1 is present on a subset of primitive hematopoietic progenitors found at various stages of development in sites of active hematopoiesis in yolk sac (Cumano et al., 1993; Auerbach et al., 1996; Yoder et al., 1997), AGM region (Godin et al., 1995; Marcos et al., 1997), fetal liver (McKearn et al., 1985; Jordan et al., 1990; Cumano and Paige, 1993), and bone marrow (Li et al., 1996; Szivassy and Cory, 1993). In yolk sac, AA4-positive cells are first detected at day 8-10 of gestation (Cumano et al., 1993; Sanchez et al., 1996). At day 9-10 of gestation AA4⁺c-Kit⁺ progenitors are found in the P-Sp/AGM region (Sanchez et al., 1996; Marcos et al., 1997), and by day 14 of development, AA4 defines 0.5-1.0% of the fetal liver tissue that contains the entire hierarchy of primitive hematopoietic cells (Jordan et al., 1990). Proliferation within each successive compartment results in increased total number of progenitor cells. Antigen density per cell also increases with developmental progress, which is especially marked for c-Kit and AA4 (Marcos et al., 1997). In bone marrow, HSC are found in both AA4⁺ and AA4⁻ subpopulations, although in adult marrow AA4 is largely regarded to be a marker of early B lymphoid lineage. In addition,

recent studies demonstrate that the expression of AA4 parallels the onset of hematopoietic development in differentiating ES cells (Kabrun et al., 1997; Lin and Neben, 1997; Potocnik et al., 1997). Taken together, these results indicate that AA4 plays an important role in hematopoiesis and has to be studied in more detail.

5 In order to achieve this goal, AA4 was molecularly cloned and characterized. The results demonstrate that AA4 is a 130kDa type I transmembrane glycoprotein whose structure suggests a role in cell adhesion. Expression analysis showed that high levels of AA4 are found in lung, heart, and bone marrow. In the hematopoietic system, expression of AA4 correlates with the expression of stem cell markers CD34 and CD43. Functional
10 studies indicate that AA4 coimmunoprecipitates with CD34 and CD43, suggesting that these proteins form a macromolecular complex which may function in the regulation of cell adhesion, proliferation and/or differentiation of hematopoietic cells.

Results

Cloning of AA4. In order to identify AA4, AA4.1 monoclonal antibody was used
15 to screen various cell lines and primary hematopoietic cells. The murine B lymphoid cell line D2N was found to express relatively high levels of AA4 antigen (see Table 3). Immunoprecipitation of protein extracts prepared from D2N cells showed that AA4.1 recognizes a protein with apparent molecular weight (M_r) 130kDa (Figure 7). This protein was also present in extracts prepared from B lymphoid cell line M2.4, and AA4
20 positive hematopoietic cells derived from bone marrow and fetal liver.

To isolate AA4, a cDNA library was prepared from D2N cells and cloned into the polylinker region of a retroviral expression vector REBNA (see Materials and Methods). Following production of retroviruses, NIH 3T3 cells were infected with the recombinant cDNA library and selected for AA4 expression by flow cytometry using PE-conjugated
25 AA4.1. After two rounds of sorting, genomic DNA extracted from AA4-positive cells was analyzed by PCR amplification using viral vector primers. This resulted in the amplification of a 3.1 kbp cDNA which was gel-purified and subcloned for further analysis. Infection of NIH 3T3 fibroblast or EML C1 hematopoietic cells with REBNA/AA4, a recombinant retrovirus expressing the cloned cDNA, has led to the
30 acquisition by cells of high affinity to AA4.1 mAb (Figure 8A). In infected cells, AA4.1 detects a 130kDa surface protein which comigrates with the endogenous AA4 from D2N cells (Figure 8B), thus indicating that the cloned cDNA encodes AA4.

Sequence analysis of AA4. Sequence analysis of the cloned cDNA (SEQ. ID No.: 72) showed that it has a single open reading frame encoding a protein of 644 amino acids (see Figure 9)(SEQ. ID No.: 73. The deduced amino acid sequence includes a putative leader peptide and the mature protein which starts at position 20. The protein
5 contains a long N-terminal extracellular region, a single putative hydrophobic transmembrane region, and 47 amino acids of the C-terminal cytoplasmic domain. The extracellular part of AA4 is composed of two major structural moieties. The N-terminal region contains a C-type lectin domain (CTL) which has 32% sequence homology to endothelial cell receptor thrombomodulin. This region is followed by a cysteine-rich
10 domain composed of six epidermal growth factor (EGF)-like repeats, three of which are consistent with the calcium-binding EGF motifs. Similar repeats are found in the extracellular domains of a large number of membrane-bound proteins and in proteins known to be secreted (Bork et al., 1996).

Database searches revealed that AA4 exhibits high homology to C1qRP, the
15 human receptor for complement component C1q expressed on surfaces of myeloid cell lineage and endothelial cells (Nepomuceno et al., 1997). Sequence alignment showed that AA4 and C1qR have approximately 68% identical amino acid positions and similar domain structures. Highest homologies were found within the N-terminal parts of the proteins and their C-terminal cytoplasmic domains (see Figure 10A and 10B), suggesting
20 that AA4 and C1qR may share functional properties.

The amino acid sequence of AA4 contains numerous potential O-linked and N-glycosylation sites. Although the predicted M_r of AA4 is 67.4 kDa, two protein bands exhibiting M_r 105kDa and 130kDa respectively, are immunoprecipitated by AA4.1 mAb in cells infected with REBNA/AA4 (Figure 8C). When ^{35}S -labeled cells are chased with
25 nonradioactive media, the intensity of the 105kDa band rapidly decreases, while the intensity of the 130kDa band increases, thus indicating that p130 is the mature form of the protein, whereas p105 is its precursor. In agreement with this conclusion, immunoprecipitation of biotinylated cells using AA4.1 reproducibly results in the detection of a 130kDa surface protein (see Figure 8B).

30 Expression patterns of AA4. Northern blot analysis showed that in adult mouse tissues, AA4 is expressed at high levels in lung, heart, and bone marrow. No detectable expression was found in brain, testis, spleen, and thymus (Figure 10B). In normal tissues

and transformed cell lines, a 7kb RNA species hybridizes with the cloned cDNA (Figure 11A and 11B). In addition to the 7kb species, poly(A)-RNA from D2N cells contains a minor band corresponding to a 3.2 kb mRNA (see Figure 11A, lane 8). Similarity search against expressed sequence tags (ESTs) showed that databases contain at least seven
5 different sequences corresponding to the 3'-untranslated region of the cloned *aa4*cDNA which were isolated from the following mouse tissues: colon (Genbank accession No AA929174), heart (AA435107), lymph node (AA185911 and AA267407), lung (AA220480), mammary gland (AI021507), and spleen (AA145088). Sequence identity with the ESTs abrogates upstream of nucleotide G at position 2481 in the 3'-untranslated
10 region of *aa4*cDNA, thus indicating that the cloned cDNA corresponds to an alternatively spliced 3.2 kb *aa4*mRNA.

Expression of AA4 in hematopoietic progenitor cells AA4 is produced in murine hematopoietic progenitor cells and immature B lymphocytes found at various stages of development in yolk sac (Godin et al., 1995; Marcos et al., 1997), fetal liver (McKearn et al., 1985; Jordan et al., 1990), and bone marrow (Cumano et al., 1992; Li et al., 1996). To
15 confirm that the cloned cDNA encodes AA4, RT-PCR was performed on hematopoietic cells fractionated using several different techniques. The cells analyzed included AA4⁺ and AA4⁻ fetal liver (FL) cells; AA4⁺ FL cells fractionated into Lin^{lo}c-Kit⁺Sca-1⁺ and Lin^{lo}c-Kit⁺Sca-1⁻ populations; and hematopoietic progenitors isolated from bone
20 marrow (BM) by cell sorting using combinations of different surface markers.

Figure 12A shows that *aa4* was amplified from AA4⁺ FL cells, whereas in AA4⁻ cells it was only present at low levels. AA4 expression was high in Lin^{lo}c-Kit⁺Sca-1⁺ cells enriched for HSC activity. In adult marrow cells, *aa4* was amplified from Lin⁺ cells and Lin⁻c-Kit⁺Sca-1⁺CD34⁺ multipotential progenitors. At lower levels *aa4* was present
25 in Lin⁻c-Kit⁺Sca-1⁺CD34⁻ long term reconstituting HSCs (Figure 12B). In all tested cells *aa4* expression correlated with the expression of stem cell markers CD34 and CD43.

Embryonic stem (ES) cells which have been shown to generate progenitors for most hematopoietic lineages during differentiation *in vitro* (Keller, 1995) were also tested in this experiment. Figure 12C shows that *aa4* was not found in undifferentiated ES cells
30 but its expression was upregulated as these cells differentiated and formed blast cell colonies (BL) and colonies of more differentiated hematopoietic cells (HMT). These results are in line with previous studies which showed that AA4 is expressed in ES-

derived hematopoietic precursors (Kabrun et al., 1997; Ling and Neben, 1997; Scott et al., 1997) and show that this expression is not abrogated upon differentiation of blast cell colonies into endothelial cells (ENT in Figure 12C).

Ectopic expression of AA4 has no mitogenic effect EML C1 and NIH 3T3 cells
5 expressing exogenous AA4 did not exhibit morphological changes indicative of alterations in their growth properties. To examine the effect of AA4 on growth phenotypes in more detail, NIH 3T3 and primary mouse embryo fibroblasts were infected with a retrovirus expressing AA4 and maintained in high and low serum conditions. FACS analysis of the transduced cells confirmed that in each case the efficiency of
10 infection was close to 100%. However, examination of growth rates showed that overexpressed AA4 had no apparent effect on proliferation of both cell types as compared to control uninfected cells or the corresponding cells infected with a retrovirus expressing GFP.

AA4 coimmunoprecipitates with CD34 and CD43 To investigate interactions
15 with other proteins, hematopoietic and fibroblast cells expressing AA4 were immunoprecipitated with AA4.1 mAb and then examined by immunoblot analysis using a panel of antibodies directed against membrane-associated proteins. This analysis revealed that AA4 coimmunoprecipitates with CD34, a membrane glycoprotein selectively expressed within the hematopoietic system on stem and progenitor cells, and CD43 which
20 is a major O-glycosylated sialomucin found on the surfaces of most leukocytes. This result is in line with previous studies which showed that CD43 coimmunoprecipitates with the human C1qRP (Guan et al., 1991; 1994). Figure 13B shows that in the murine D2N and EML C1 cells, a 52kDa protein is the major isoform of CD43 that associates with AA4. This 52kDa protein was found to be reactive with both the N-terminal (S19)
25 and C-terminal (M19) anti-CD43 antibodies, indicating that it is not a breakdown product. In NIH 3T3 fibroblasts coexpressing CD43 and AA4, a 54kDa and a 170kDa CD43 isoforms coimmunoprecipitated with AA4 pointing to the glycosylation differences between CD43 expressed in different cell types. A 115kDa CD43 isoform which
30 previously have been shown to be sialylated and thus overly negatively charged (Guan et al., 1994) did not form macromolecular complexes with AA4 in NIH 3T3 cells. Similarly, AA4 did not coimmunoprecipitate with a 115kDa CD43 isoform found in D2N cells and a 120kDa isoform found in EML C1 (Figure 13B).

To examine associations of AA4 with CD34, both proteins were expressed in Rat-1 cells following infection with the corresponding retroviruses. See Figure 13C.

In Rat-1 cells coexpressing AA4 and CD34, AA4.1 the results also demonstrate that AA4 coimmunoprecipitates with CD34. Studies have suggested a role for both CD34 and CD43 in the regulation of adhesion, growth and differentiation of hematopoietic precursors (Ardman et al., 1992; Bazil et al., 1997; Chen et al., 1996; Suzuki et al., 1996; Wood et al., 1997; Stockton et al., 1998). Coexpression of AA-Fc fusion protein in NIH 3T3 cells stably producing AA4 showed that AA4-Fc forms heterodimeric complexes with AA4. Figure 11A shows that equimolar amounts of AA4 and AA4-Fc were coprecipitated from these cells by protein A, indicating that AA4 is prone to homo- or heterodimerization.

Discussion

This Example describes the expression cloning and sequence analysis of AA4, a molecular marker expressed on hematopoietic stem and progenitor cells. The CDNA encoding AA4 was isolated from a retroviral cDNA library prepared from the murine D2N lymphoid cell line. Sequence analysis of the cloned cDNA revealed that AA4 is a type I transmembrane protein composed of 625 amino acids. The extracellular part of the molecule contains two major structural moieties, a C-type lectin carbohydrate recognition domain and six EGF-like domains. Similar repeats have been found in a large number of membrane-bound proteins or in proteins known to be secreted. The cytoplasmic domain of AA4, in contrast, bears no structural similarity with known protein families. Instead, AA4 revealed strong homology to ClqR, the human receptor for complement component Clq which is predominantly expressed in phagocytic cells such as monocytes, neutrophils, and endothelial cells (Nepomuceno et al., 1997). Sequence alignment shows that AA4 and ClqR have approximately 68% identical amino acid positions and share similar domain structure. Highest homologies were found within the Nterminal parts of the two proteins and their C-terminal cytoplasmic domains, suggesting that AA4 and ClqR may have similar functional properties. Expression of AA4 correlates with the expression of CD34 and CD43, two other surface markers normally present on murine hematopoietic stem and progenitor cells. Previous studies have shown that AA4-positive cells are first detected in yolk sac at day 8-10 of gestation (Cumano et al., 1993; Sanchez et al., 1996).

At day 9-10 pc AA4+c-Kit+Mac-1+ progenitors are found in the P-Sp/AGM region (Sanchez et al., 1996; Marcos et al., 1997), and by day 14 of gestation, AA4

defines 0.5-1.0% of the fetal liver tissue that contains the entire hierarchy of primitive hematopoietic cells (Jordan et al., 1990). As proliferation within each successive compartment results in increased total number progenitor cells, antigen density per cell also increases with developmental progress, which is especially marked for c-Kit and AA4 (Marcos et al., 1997). A significant proportion of hematopoietic progenitors from yolk sac also express CD34, CD38, CD43, CD44, and Mac-1 however show little or no expression of CD4, CD8, CD45R and Sca-. These same markers are present on FLderived HSC which also express Sca-1. Adult marrow HSCs also express cKit, CD38 and Sca-1, but do not normally express Mac-I or AA4. In BM, HSC are found in both AA4+ and AA4-subpopulations, although in adult marrow AA4 is largely regarded to be a marker of early B lymphoid lineage 0. These results indicate that the expression of cell surface antigens changes on HSC during ontogeny and that differential display of these cell surface markers may reflect relationship between HSCs that contribute to multilineage hematopoiesis and distinct anatomical sites during development.

15 **Methods**

Cells and tissue culture. NIH 3T3 fibroblasts were grown in DME medium supplemented with 10% fetal calf serum (FCS). D2N cells were grown in RPMI 1640 medium containing 10% FCS. EML Cl cells were grown in IMDM supplemented with 20% horse serum and 8% BHK/MKL conditioned medium (Tsai et al., 1994). To maintain the multipotentiality of EML Cl, the cells were kept at low density (0.5 - 5x10⁵/ml) and subcultured every two days. Cell lines constitutively expressing AA4 and GFP were derived from NIH 3T3 or EML Cl cells by infecting with the corresponding recombinant retroviruses.

Retroviral-mediated gene transfer. Retrovirus expression vector REBNA was constructed by substituting the LacZ gene contained within the EcoRI-NotI fragment of plasmid LZRSBPMN-Z (Kinsella and Nolan, 1996) with a synthetic polylinker composed of EcoRI, XhoI, SfiI, and NotI sites. Retroviral vector REBNA/IRESGFP contains a poliovirus IRES element and the CDNA encoding color-enhanced GFP (S. Zolotukhin, Gainesville, FL) inserted into REBNA.

For DNA transfections, cells were plated at a density 2x10⁶ cells per 60mm dish and transfected with 5ug of plasmid DNA using 20ul of lipofectamine reagent (GibcoBRL). REBNA-transfected cells were selected in puromycin (2ug/ml) and grown to confluence prior to collecting virus supernatant. For infections" the culture medium

was supplemented with polybrene (Sigma) at 5ug/ml. The appropriate virus was added and incubated overnight. Multiple *infections were carried* sequentially, with the *appropriate* selection between.

Isolation o.t RNA and CDNA cloning. Poly(A) RNA prepared from D2N cells
5 was converted into CDNA using Superscript II Reverse Transcriptase (GibcoBRL) and an
oligo(dt) primer containing NotI site, 5'-TGGTGTCTGACGACGAGTAGCGGCCGCT18
(SEQ.ID.No.:74). The second strand was synthesized using DNA polymerase I in
combination with *E. coli* RNase H and *E. coli* DNA ligase as described (Gubler and
Hoffman, 1983). An adaptor composed of complementary oligonucleotides, 5'-
10 GGCCCGGGCCGGCC (SEQ.ID.No.:75) and 5'-TCGAGGCCGCGCCGGGCC
(SEQ.ID.No.:76), was ligated to the CDNA and cut with NotI to produce CDNA
molecules with NotI and XhoI termini for directional cloning. After size fractionation in
agarose gel, cDNAs larger than 2.5 kbp were ligated into NotI and XhoI cut plasmid
REBNA and electroporated into electrocompetent DH12S cells (GibcoBRL). Plasmid
15 DNAs were transfected into 293-derived packaging cell line for retrovirus production.
Virus-containing supernatants were collected and stored at -80C.

NIH 3T3 cells infected with the recombinant retroviruses representative of D2N
CDNA library were selected for the production of AA4 by flow cytometry using
phycoerythrin-conjugated AA4.1 mAb. After two rounds of sorting, genomic DNA
20 isolated from AA4-positive cells was subjected to pcr amplification using retroviral
vector primers, 5'CAGCCCTCACTCCTTCTC (SEQ.ID.No.: 77) and 5'-
GGTGGGGTCTTTCATTCC (SEQ.ID.No.: 78) (Kitamura et al., 1995). Amplified
CDNA was gel purified and subcloned into pbluescript SK and REBNA plasmid vectors.
Nucleotide sequences were analyzed using NCBI Blast database search programs and
25 ExPASy molecular biology server from the Swiss Institute of Bioinformatics.

Northern blot hybridization. RNAs prepared using acid guanidinium thiocyanate-
phenol extraction procedure (Chomczynski and Sacchi, 1987) were separated on
formaldehydeagarose gels and blotted onto the Hybond-N nylon membranes
(Amersham). Hybridization probes were derived from cloned cDNAs using Ready To
30 Go DNA labeling beads (Pharmacia Biotech). Hybridizations were performed as
described previously (Petrenko et al., 1997).

Cell labeling and Immunoprecipitations. The ECL protein biotinylation system
(Amersham) for the detection of cell surface proteins was used as recommended by the

manufacturer. For radioactive labeling, 2×10^6 cells were incubated with 200 μ Ci Translabel (ICN) in 2ml of cysteine and methionine-deficient medium for 2hr at 37°C. Cells were washed in PBS and lysed 10min. on ice in 500 μ l of NP40 buffer containing 20mM TrisHCl pH 7.6; 150mM NaCl; 0.5% NP40; 1mM PMSF; 5mM benzamidine; 1mM sodium vanadate; 10 μ g/ml aprotinin; 10 μ g/ml leupeptin. Lysates were cleared by centrifugation and 2 μ g of soluble antibody were added to the supernatants followed by 25 μ l of Protein G-Sepharose suspension (Sigma). After 2 to 4hr incubation at 40°C with rotation, protein G-antibody complexes were pelleted and washed in the successive changes of wash buffer 1 (1M NaCl; 10mM TrisHCl pH 8; 0.1% NP40); wash buffer 2 (0.1M NaCl; 10mM TrisHCl pH 8; 0.1% NP40); wash buffer 3 (10mM TrisHCl pH 8; 0.1% NP40). Samples were eluted by boiling 2min. in 2Xgel loading buffer, separated by SDS/PAGE, dried and exposed to X-ray film.

Western blot analysis. Protein extracts for Western blot analysis were prepared as described (Morrison et al., 1991). The antibodies used included goat anti-mouse CD43 polyclonal IgG (M19 and S-19, Santa Cruz) in combination with HRP-conjugated secondary antibodies and ECL detection system (Amersham).

Flow cytometry and RT-PCR. Timed-pregnant mice and 5- to 7-week-old female mice (C57BL/6j) were purchased from Jackson Laboratory (Bar Harbor, ME). AA4-positive cells were isolated from day 14 fetal liver by immunopanning on Petri dishes coated with AA4.1 antibody (10 μ g/ml). Hematopoietic stem cells were purified from AA4-positive fraction by staining with lineage-specific antibodies as described previously (Moore et al., 1997). Three-color fluorescence-activated cell sorting for lineage negative to low, Sca-1(+), c-Kit(+) cells was performed on a multilaser FACS Vantage with CellQuest software (Beckton Dickinson). ES cells differentiated into blast cell colonies, hematopoietic progenitors, and endothelial cells prepared as described previously.

For RT-pcr, poly(A)-RNA isolated from sorted cells was converted into cDNA using Superscript II Reverse Transcriptase (GibcoBRL) and CapFinder cDNA amplification kit (Clontech). Gene-specific primers for pcr amplification included: 5'-TTCAGCAAGCCCTGACTC (SEQ.ID.No.:79) and 5'-GCCACCTTCGAAGCAATC (SEQ.ID.No.:80) (AA4); 5'-GAGCGGTACAGGAGAATG (SEQ.ID.No.:81) and 5'-GCCCACCCAACCAAATCA (SEQ.ID.No.:82) (CD34); 5'-ACCGCGTTCTTCTGTAAC (SEQ.ID.No.:83) and 5'-CAGCTAACAGCAGGATCC

(SEQ.ID.No.:84) (CD43); G3PDH Control Amplimer Set (Clontech) for the amplification of GAPDH.

**EXAMPLE 3: *IN VITRO* MAINTENANCE OF HIGHLY PURIFIED,
TRANSPLANTABLE HEMATOPOIETIC STEM CELLS**

The cellular and molecular mechanisms which regulate even the most primitive
5 hematopoietic stem cell are not well understood. This example details a systematic
dissection of the complex hematopoietic microenvironment in order to define some of these
mechanisms. An extensive panel of immortalized stromal cell lines from murine fetal liver
was established and characterized. Collectively, these cell lines display extensive
heterogeneity in their *in vitro* hematopoietic supportive capacities. This example describes a
10 long-term *in vitro* culture system, utilizing a single, stromal cell clone (AFT024) that
qualitatively and quantitatively supports transplantable stem cell activity present in highly
purified populations. Disclosed is multi-lineage reconstitution in mice that received the
equivalent of as few as 100 purified bone marrow and fetal liver stem cells which were
cultured for 4-7 weeks on AFT024. The cultured stem cells meet all functional criteria
15 currently ascribed to the most primitive stem cell population. The levels of stem cell activity
present after 5 weeks of coculture with AFT024 far exceed those present in short-term
cytokine-supported cultures. In addition, the maintenance of input levels of transplantable
stem cell activity is accompanied by the expansion of other classes of stem/progenitor cells.
This suggests that the stem/progenitor cell population is actively proliferating in culture and
20 that the AFT024 cell line provides a milieu which stimulates progenitor cell proliferation
while maintaining *in vivo* repopulating activity.

Introduction

Mammalian blood formation originates in a small population of hematopoietic stem
cells. The hallmark features of these cells are: (1) a hierarchical multilineage differentiation
25 potential with the ability to clonally give rise to at least 8 distinct cell lineages, (2) self-
renewal capacity which is reflected in the life long continuous activity of few, in some cases
single, stem cells and (3) a dramatic proliferative potential which is ultimately responsible
for the production of large numbers of mature blood cells. (Lemischka, I.R. 1992;
Morrison, S.J. et al. 1995; Harrison, D.E. 1980). During the past decade much progress has
30 been made in providing a physical phenotype for this rare population of stem
cells.(Spangrude, G.J., et al. 1995; Lemischka, I.R. 1992; Bauman, J.G., et al. 1998).
However, currently, the only reliable functional assay system for the most primitive stem
cell compartment is long-term *in vivo* transplantation. No *in vitro* system has been

developed which adequately recapitulates stem cell behaviors. Therefore, the cellular and molecular mechanisms that regulate the biology of stem cells have remained obscure.

A major challenge in stem cell research is the establishment of culture systems which facilitate *in vitro* maintenance of long-term transplantable stem cell activity. This is a
5 necessary first step towards a cellular and molecular understanding of the regulatory mechanisms which mediate commitment versus self-renewal decisions. Moreover, the establishment of such culture systems is a prerequisite for the potential expansion of undifferentiated stem cell populations as well as for the generation of stem/progenitor cells committed to selected lineages.

10 Efforts to develop culture systems for the maintenance of transplantable stem cells can be subdivided into two broad categories: (1) those utilizing defined cytokine combinations as the only culture supplements and (2) those relying on a pre-established stromal monolayer as an additional supportive component (with or without exogenously added cytokines). Both of these strategies have met with only limited success. In the first
15 case, it has been repeatedly demonstrated that combinations of cytokines can exert potent stimulatory effects on stem/progenitor populations. (Spangrude, G.J., et al. 1988; Jordan, C.R. et al. 1990; Fleming, W.H., et al. 1993). In some studies, highly purified stem cells (Li, C.L., and Johnson, G.R. 1990; Spangrude, G.J. and Johnson, G.R. 1990). have been used and the direct effects of cytokines have been demonstrated at the single cell level. (Jones, R.
20 et al. 1990; Uchida, N. et al. 1993). While informative, the vast majority of these studies are limited by their strictly *in vitro* nature. Thus, it is feasible to expand, replatable *in vitro* progenitor populations (Li, C.L., and Johnson G.R. 1992; Uchida, N. et al. 1993) and to stimulate colony-formation by cells with both myeloid-erythroid and lymphoid potentials, Jones, R., et al. 1990; Uchida, N. et al. 1993; Trevisan, M., and Iscove, N.N. 1995; Ogawa,
25 M. 1993) however, the equivalence of these progenitor cells with the *in vivo* transplantable stem cell population remains speculative. Several studies have clearly demonstrated a dramatic loss of *in vivo* repopulating potential as a result of cytokine driven *in vitro* proliferation.(Knobel, et al., 1994; Peters, et al., 1995; Traycoff, et al., 1996). A small number of studies have shown that defined cytokine combinations promote the maintenance
30 of transplantable activity.(Rebel., et al., 1994). However, most of these are limited both by the use of very short culture periods, the exact nature of the *in vivo* assay, and the use of non-enriched stem cell sources.(Muench, et al., 1993; Holyoake, et al., 1996; Soma, et al.,

1996). This precludes interpretations suggesting a direct action of the given cytokine(s) in maintaining transplantable activity.

A further complication with defined cytokine studies is the inability to ascribe *in vivo* physiological relevance to the observed effects. It has long been accepted that in the intact
5 animal, stem cells are found in close association with discrete cellular microenvironments.(Lord, et al., 1975; Trentin, et al., 1970; Weiss, et al., 1991; Wolf, 1979). These observations suggest both the existence of stem cell niches and the notion that *in vivo* stem cell regulatory mechanisms are likely to require cell-cell contact or short range interactions.(Dorschking, 1990). Efforts to understand the features of the hematopoietic
10 microenvironment began with the establishment of the Dexter long-term culture (LTC) system. (Dexter, et al., 1977). In this culture system hematopoiesis is maintained for weeks or months by a heterogeneous adherent cell monolayer derived from bone marrow (BM). While some degree of transplantable stem cell maintenance and self renewal (Fraser, et al., 1990) has been demonstrated, a general feature of the Dexter-LTC is a dramatic net decrease
15 of stem cell activity over time. (Harrison, et al., 1987; Van der Sluijs, et al., 1993). Although much progress has been made, especially in studies of human stem/progenitor cells, (Sutherland, et al., 1989; Verfaillie, et al., 1995; Hao, et al., 1996) a further drawback of this system is the heterogeneity of the stromal cell types present in the supportive monolayer. This hampers the identification of regulatory mechanisms. Studies have been reported
20 where the heterogeneous stromal monolayer is replaced with cloned stromal cell lines. (Roberts, et al., 1987; Kodama, et al., 1984; Issad, et al., 1993; Wineman, et al., 1993). Many of these cell lines can support *in vitro* myelopoiesis, (Suzuki, et al., 1992; Neben, et al., 1993; Kodama, et al., 1992) B-lymphopoiesis (Collins, et al., 1987; Whitlock, et al., 1982) or in some cases both.(Wineman, et al., 1993; Wineman, et al., 1996) However, very few
25 studies have focused on the *in vitro* maintenance of the most primitive transplantable stem cell compartment. Moreover, with one exception (Szilvassy, et al., 1996) the studies which have focused on the *in vitro* maintenance of this stem cell population begin with heterogeneous unpurified sources of hematopoietic activity. Wineman, et al., 1992, 1996; Deryugina, et al., 1994). Such populations contain numerous non-hematopoietic stromal cell
30 types. Therefore, it has not yet been possible to assign a direct stem cell supporting phenotype to a given stromal cell line.

It was hypothesized that the rare frequency of primitive, stem cells may suggest an equally rare frequency of stem cell supporting microenvironmental niches. Accordingly, we

established and characterized a large panel of conditionally immortalized, cloned stromal cell lines from mid-gestation fetal liver. This organ was chosen because, during development, it is here that the stem cell compartment is undergoing self-renewal expansion in addition to differentiation. (Moore, et al., 1970). The cell lines were generated as previously described, (Wineman, et al., 1996) by immortalization with a temperature sensitive SV40TAg. (Frederiksen, et al., 1988) The clonal nature of the AFT024, 2018, and 2012 cell lines was verified by Southern blot analysis which detected a single, unique proviral integration locus in their genomic DNA. In order to identify potentially interesting cell lines, we used a "cobblestone area" (CA) assay (Ploemacher, et al., 1989) was initiated with BM taken from mice injected two days previously with 5-fluorouracil (5-FU). It has been suggested that CA colonies which appear after a prolonged culture period are derived from more primitive stem cells, possibly identical to some *in vivo* transplantable entities. (Ploemacher, et al., 1991). Therefore, a goal was in identifying cell lines which support such late arising CAs. Of 225 lines, 77 (34%) were capable of supporting limited *in vitro* hematopoiesis, while, consistent with the initial hypothesis, only 2% were able to maintain long-term (>6 weeks) hematopoietic CA activity. Subsequent studies with a selected subset of these lines, showed that the ability to effectively support *in vivo* reconstituting BM stem cells is infrequently observed. (Wineman, et al., 1996). Two out of sixteen cell lines maintained significant levels of long-term reconstituting stem cell activity for an *in vitro* culture period of three weeks. Several other cell lines supported low levels of such activity or transiently repopulating stem cells. The cell inoculum, in these studies, was whole BM which was not enriched for stem cell activity. Therefore, it was not possible to suggest that the effective stromal cell lines were directly supporting stem cell activity. This example demonstrates that a single clonal cell line, designated AFT024, can maintain quantitative levels of transplantable stem cell activity present in highly purified stem cell populations. These data were generated using a competitive repopulation assay system, which employs uncompromised competitor BM cells. The *in vitro*-maintained stem cells satisfy all criteria which currently define the most primitive stem cell population including the ability to reconstitute secondary recipients. This example also shows that the *in vitro* maintenance of primitive transplantable stem cells is compatible with the concurrent generation of large numbers of committed progenitors.

Methods

Mice. Timed-pregnant mice and 5-7 weeks old females (C57Bl/6J, Ly5.2) were purchased from the Jackson Laboratory (Bar Harbor, ME). Congenic C57Bl/6, Ly5.1 female mice were purchased from the National Cancer Institute (Frederick, MD). All mice
5 were housed in the Princeton University Barrier Animal Facility, in autoclaved micro-isolator cages on ventilated cage racks. The animals received sterile, irradiated food, and acidified, autoclaved water ad libitum.

Stromal cell lines and culture conditions. The fetal liver stromal cell lines used in this study were derived as previously described. (Wineman, et al., 1996). Stromal cell lines
10 were routinely cultured in Dulbecco's modified Eagles's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 5×10^{-5} mol/L b-mercaptoethanol (2-ME), at 32°C, 5% CO₂, 100% humidity. Sera were obtained from Hyclone, Logan, UT. Other biochemical reagents were obtained from Sigma, St. Louis, MO. Two of the lines used in this study (2012 and 2018) were previously characterized for their ability to support long-term
15 repopulating activity present in whole unfractionated BM. (Wineman, et al., 1996). The AFT024 cell line was identified as an additional long-term (>4 weeks) CA supporter. Subclones of 2012 and AFT024 were isolated and used in these studies. The AFT024 cell line has remained stable and demonstrated consistent stem cell supporting abilities for over 4 years.

Hematopoietic stem cell purification. Stem cells were purified from day 14 fetal
20 livers essentially as described, (Jordan, et al., 1990) with the inclusion of c-kit expression as an additional parameter. Briefly, AA4.1⁺ cells were isolated by immunopanning on petri dishes coated with AA4.1 antibody (10 ug/mL). The AA4.1⁺ fraction has been shown to contain all repopulating stem cell activity present in day 14 fetal liver. (Jordan,
25 et al., 1990) AA4.1⁺ cells were collected and stained with saturating concentrations of fluorescein isothiocyanate (FITC) labeled rat monoclonal antibodies to lineage markers (CD3, CD4, CD5, CD8, B220, Gr-1, Mac-1, and TER-119). The cells were simultaneously stained with phycoerythrin (PE) labeled Ly6A/E (Sca-1) antibody and biotinylated antibody to c-kit. The latter was developed with streptavidin
30 allophycocyanin (APC). The AA4.1 hybridoma was a kind gift from Dr. J. McKearn, Monsanto, St. Louis, MO. AA4.1 antibody was purified by ImClone Systems Inc. New York, NY. The TER-119 antibody was initially obtained from Dr. T. Kina, Kyoto University, Japan and subsequently purchased from PharMingen, San Diego, CA. All

other antibodies were purchased from PharMingen. Streptavidin APC was purchased from Molecular Probes Inc., Eugene, OR. Three color fluorescence activated cell sorting for lineage negative to low ($\text{lin}^{-/\text{lo}}$), Sca-1⁺, c-kit⁺ cells was initially done on a dual laser Epics 753 cell sorter (Coulter Electronics, Hialeah, FA) interfaced with Cicero software
5 (Cytomation Inc., Fort Collins, CO) and subsequently on a multi-laser FACS Vantage with Cell Quest software (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Enriched fractions of BM stem cells were obtained from C57Bl/6 Ly5.1 mice as described. (Okada, et al., 1992). Briefly, BM mononuclear cells were isolated by density centrifugation over Ficoll Hypaque (<1.077, Pharmacia, Piscataway, NJ). Lineage
10 negative or low staining cells ($\text{lin}^{-/\text{lo}}$) were obtained by magnetic bead depletion (anti-rat immunoglobulin coated beads, Dynal, Oslo, Norway) of mononuclear cells using the same lineage cocktail described herein above. The cells were further stained with antibodies to Sca-1 and c-kit as described herein above. Sorting for $\text{lin}^{-/\text{lo}}$, Sca-1⁺, and c-kit⁺ cells was accomplished with the Epics 753 as described above.

15 Stem cell/stromal cell cocultivation and cytokine-supplemented suspension culture. Stromal cell lines were seeded on tissue culture dishes that had been coated with 1% gelatin (Specialty Media, Lavallete, NJ) and were grown at 32⁰C, 5% CO₂, 100% humidity. Confluent monolayers were irradiated (20 Gy, ¹³⁷Cesium source, Gammacell 40, Nordion International Inc. Ontario, Canada) and cultured in modified Dexter (Dexter,
20 et al., 1984) media (DMEM, 10% FBS, 10% horse serum, 5X10⁻⁵ mol/L 2-ME, 1X10⁻⁷ mol/L hydrocortisone). For Dexter-LTC, enriched hematopoietic stem cells were added and the cultures were maintained at 37⁰ C, 5% CO₂, 100% humidity with weekly media changes. The specific numbers of purified stem cells added to stromal cell cocultures are given in the appropriate figure or table legends. In some experiments, week 4
25 AFT024/stem cell cultures were harvested and replated in limiting-dilution onto fresh, irradiated (20 Gy) AFT024 monolayers in 96-well trays (Dexter-LTC conditions). CAs were scored weekly (as described above), for an additional 5 weeks. Irradiated (20 Gy) 2018 monolayers in 96-well trays were used in limiting-dilution Whitlock-Witte assays (LD-WW) (Whitlock, et al., 1982) to assess stromal-dependent B-lymphopoiesis content
30 of both freshly purified and AFT024 cultured fetal liver stem cells. These cultures were established in RPMI media with 5% FBS, 2 mmol/L glutamine, 1 mmol/L Na pyruvate, and 5X10⁻⁵ mol/L 2-ME at 37⁰ C, 5% CO₂, 100% humidity. 2018 has been identified as a potent B-lymphopoiesis supporting line in W-W conditions. (Deryugina, et al., 1994).

Short-term cytokine-supported suspension cultures and short-term AFT024/stem cell cocultures were established in Iscove's Modified Dulbecco's Media (IMDM), 10% FBS, 1% BSA, 5×10^{-5} mol/L 2-ME at 37°C, 5% CO₂, 100% humidity. Cytokine concentrations: rmflk2/flt3-ligand (FL) 30 ng/mL, rmSteel factor (SL) 20 ng/mL, rhIL-6
5 10 ng/mL. FL was obtained from ImClone Systems Inc.; SL was purchased from Genzyme Corporation, Cambridge, MA. IL-6 was purchased from Upstate Biotechnology Inc., Lake Placid, NY.

Transplantation assays for hematopoietic stem cell activity. Competitive repopulation was used to measure stem cell activity present in both freshly isolated and cultured stem
10 cell populations. (Harrison, et al., 1993). This assay was performed using the congenic Ly5.1/5.2 mouse system (Morse, et al., 1987). Enriched stem cells were seeded onto irradiated stromal monolayers and maintained in Dexter-LTC conditions. At the end of 4-7 weeks, the cultures were harvested by vigorous trituration. Single cell suspensions were prepared by passage through 22-gauge needles, mixed with fresh congenic BM and
15 transplanted into lethally irradiated congenic mice (10 Gy, split dose 3 hours apart, 1 Gy/min, Gammacell 40). All purified fetal liver stem cells were from Ly5.2 mice. The competitor BM and recipients were Ly5.1. Purified BM cells were from Ly5.1 mice; in this experiment, Ly5.2 BM was used as competitor and Ly5.2 mice were used as recipients. In order to assess reconstitution, mice were periodically bled by capillary
20 puncture of the orbital venous plexus. Blood (0.1 mL) was collected into heparin-containing (10 U/mL) DMEM and the red blood cells were lysed with NH₄Cl (Mishell, et al., 1980). For the experiments described in Figure 15 and Table 4., the nucleated cells were divided into two fractions and stained with the appropriate biotinylated Ly5 antibody and developed with streptavidin-peridinin chlorophyll protein (PerCP) and; (1)
25 CD4-FITC, CD8-PE and (2) B220-FITC, Mac-1-PE, Gr-1-PE. Cells from each fraction were analyzed on an Epics Profile II, Coulter Electronics. For the experiments described in Figure 16 and Tables 2. and 3., nucleated cells were stained with directly conjugated lineage antibodies (CD4-PE, CD8-PE, Mac-1-FITC, Gr-1-FITC, and B220-APC) and biotinylated Ly5.2 antibody which was developed with streptavidin Texas Red (T.R.).
30 Four color analysis of stained cells was performed on either the Coulter Epics 753 or Becton Dickinson FACS Vantage with the appropriate software interfaces described above. Anti-Ly5.1 was a kind gift of Dr. H. Nakauchi, University of Tsukuba, Japan. Purified and biotinylated Ly5.2 antibody was originally obtained from a hybridoma

(AL14A2) kindly provided by Dr. G. Spangrude, University of Utah Medical Center, Salt Lake City, UT. In later experiments, the Ly5.2 antibody was purchased from PharMingen; CD4-PE, CD8-PE, and B220-APC were also purchased from PharMingen. Streptavidin-PerCP was purchased from Becton Dickinson Immunocytometry Systems. Streptavidin T.R. was purchased from Molecular Probes. Competitive repopulating units (CRU) per 10^5 were calculated according to the formula: (Harrison, et al., 1990).

$$\text{CRU}/10^5 = \frac{\% \text{Ly5 positive cells}}{100 - \% \text{Ly5}} \times \frac{\text{cell number competitor BM}}{\text{number of test cells}}$$

10

Reconstitution values of less than 2% of the test Ly5 donor allele were not considered sufficiently above background for calculation of CRU.

Retransplantation potential of stem cells was assessed by secondary transplantation. Mice from the experiment presented in Figure 14. were sacrificed 60 weeks after transplantation, BM was harvested and stained with antibody to Ly5.2 followed by streptavidin T.R. Ly5.2⁺ cells were collected by cell sorting (Coulter Epics 753) and used to transplant lethally irradiated secondary Ly5.1 recipients. Marrow from mice in the Control and AFT024 groups was used to transplant mice in both radioprotection and competitive repopulation assays. Ly5.2⁺ BM from the primary 2012 transplants was used only in radioprotection assays. Primary 2018 mice did not contain sufficient Ly5.2⁺ cells for secondary transplantation. Transplanted mice were bled and analyzed by 4-color flow cytometry for the presence of Ly5.2⁺ cells and multilineage reconstitution as described above.

In vitro hematopoietic progenitor cell assays. The progenitor content of freshly purified hematopoietic stem cell populations and AFT024/stem cell cocultures was assessed using a variety of *in vitro* assays. All of the following assays were accomplished with fetal liver stem cells enriched as described herein above. To determine the time course of CA development, enriched stem cells were seeded onto irradiated AFT024 monolayers (300-600 cells/well in 12-well trays). CA development was followed over time and characteristic clusters were quantitated as described above. At different time points of the stem cell/AFT024 cultures, individual wells were harvested and replated into cytokine-supplemented semisolid clonogenic progenitor assays (CFU-C). The cytokine-enriched (rmIL-3 10 ng/mL, rhIL-6 10 ng/mL, rmSL 50 ng/mL, Epo 3 U/mL) methyl-

cellulose mixture was purchased from Stem Cell Technologies Inc., Vancouver BC, Canada. Colonies were scored after 8-14 days of culture at 37⁰, 5% CO₂, 100% humidity according to established criteria. (Testa, et al., 1993). Colonies that reached >1mm in size after 8 days and which contained erythroid bursts and multiple myeloid cell lineages including megakaryocytes were scored as high-proliferative potential-mixed lineage colonies (CFU-HPP-Mix). (Lowry, et al., 1995). Lineage content of typical colonies was determined by Wright's/Giemsa staining of cytopsin slide preparation from individual colonies. Colony assays were also done with 10³ freshly purified cells. The CFU progenitor contents of the AFT024 cocultures were normalized to an initial input of 10³ stem cells. To assess the ability of the AFT024 cell line to maintain primitive lymphoid progenitors, 4 week cocultures were plated into LD-WW assay on 2018 cells as described above. Resulting pro-B cell colonies were scored after 7 days. The cell number in individual wells (96-well trays, 8 wells/cell number) was normalized from the original number of purified stem cells that initiated the coculture, i.e. stem cell equivalents/well. As calculated from the line of best fit, the cell number at 37% negative wells is the frequency of pro-B cell colony initiating cells in the starting population. (Taswell, 1981). In a similar manner, the frequency of CA initiating cells in week 4 stem cell/AFT024 cocultures was also determined by replating them in limiting-dilution onto fresh, irradiated AFT024 monolayers in 96-well trays. CA were scored as described above, at 1, 2, 3, 4, and 5 weeks after replating in Dexter-LTC. The resulting frequencies were calculated as described herein above for the LD-WW assays and are also expressed in relationship to the number of stem cells that seeded the initial cultures (stem cell equivalents).

Results

In vivo and in vitro assays for stem cell activity maintained by stromal cell lines. Highly enriched stem cell populations were used to initiate cultures supported by single stromal cell lines. The focus was on one cell line, AFT024, which exhibited particularly potent stem/progenitor cell support. Two other stromal cell lines, 2012 and 2018, (Wineman, et al., 1996) were included in some experiments. In order to more rigorously establish the clonality of these lines, they were subcloned by limiting-dilution. All subclones obtained from a given cell line contained the same proviral integrant position as the parental cell line. The AFT024 cell line was evaluated both for its ability to maintain *in vivo* competitive repopulating stem cells, as well as a broad spectrum of

stem/progenitor cells defined by a variety of *in vitro* assays. The *in vivo* assays focused on the ability of stem cells, cultured for extended time periods (4-7 weeks), to permanently reconstitute multi-lineage hematopoiesis in transplanted hosts. The *in vitro* assays included the enumeration of CA appearing over time in the initial cultures as well as the quantitation of stem/progenitor cells which can form colonies in cytokine-supplemented replating assays. Cells from four-week AFT024/stem cell cocultures were also assayed by limiting-dilution for the content of progenitors capable of initiating secondary CAs on AFT024 or B-lymphopoiesis in Whitlock-Witte cultures supported by 2018.

10 AFT024 maintains quantitative levels of long-term *in vivo* repopulating stem cell activity. One line of investigation inquired if and at what levels *in vivo* transplantable stem cell activity was present in four to seven week-old cultures initiated with highly enriched stem cells and supported by AFT024, 2012 or 2018. Purified day 14 fetal liver cells (AA4.1⁺, lin^{-lo}, Sca-1⁺, c-kit⁺) and adult BM cells (lin^{-lo}, Sca-1⁺, c-kit⁺) were used as
15 sources of stem cell activity. Both of these populations are about 1000 to 1500-fold enriched for stem cell activity, as measured by competitive repopulation. (Harrison, et al., 1993). The Ly5.1/Ly5.2 congenic system was utilized for all competitive repopulation studies. (Morse, et al., 1987). The data presented in Figure 14 demonstrate that the cultures supported by AFT024 contain stem cell activity at levels quantitatively identical
20 to those present in the uncultured purified populations. In this experiment, individual Ly5.1 mice received 10³ freshly purified Ly5.2 cells or the cultured equivalent of 10³ purified Ly5.2 cells. Each mouse also received 10⁶ Ly5.1 competitor BM cells. The percentage of Ly5.2 positive peripheral blood cells was approximately equal in both groups of recipient animals. Moreover, the cultured stem cell activity is as effective as
25 freshly purified activity for *in vivo* periods of greater than one year. The data in Figure 14 also show that the 2018 cell line is completely ineffective in maintaining highly purified stem cell activity while the 2012 cell line supports intermediate levels of repopulating activity. The data presented in Table 4A. provide quantitative competitive repopulating unit (CRU) value calculations as well as the results of multiparameter lineage analyses.
30 The extremely low levels of reconstitution by 2018-cultured stem cells precluded lineage analysis. The CRU values of the AFT024-cultured and freshly purified populations are nearly identical. Moreover, both fresh and AFT024-cultured stem cells reconstitute myeloid and lymphoid cell populations to a similar degree. In order to further access the

supporting activities of AFT024 and 2012 we utilized a 10-fold lower number of fetal liver stem cells from two separate purifications to initiate the cocultures. The cultures were continued for 4-7 weeks, harvested, and used in competitive repopulation studies. Each recipient received the cultured equivalent of 100 purified Ly5.2 stem cells plus
5 4×10^5 Ly5.1 competitor BM cells. A total of twelve mice were transplanted with AFT024 cocultures (four each after four, five and seven weeks of LTC). The parental AFT024 line was used in the 4 week group and two different subclones were used to support the five and seven week cultures. The AFT024-cultured Ly5.2 stem cells contributed to 20-30% of peripheral blood cells in these recipients while cells cultured on
10 2012 demonstrated more limited *in vivo* function (Table 4B). The 2012 cultures were done with two subclones of the parental line and were maintained for four weeks prior to harvest and transplant (four mice/subclone). The data utilizing different cultures time or subclones did not vary significantly from each other and are presented together in Table 4B. An additional experiment was undertaken using enriched BM which was cultured on
15 AFT024 and 2018 for six weeks. In this experiment, BM was purified from Ly5.1 congenic mice. Each Ly5.2 recipient mouse in this study received 100 freshly purified cells, or the cultured equivalent of 100 purified cells. Both groups received 10^5 Ly5.1 competitor BM cells per mouse. Data analysis, for the presence of Ly5.1⁺ cells at 4 months after transplant, is presented in Table 4C. For 6 weeks of culture, AFT024 cells
20 maintained quantitative levels of reconstituting activity present in 100 purified BM stem cells. The 2018 cell line failed to maintain stem cell activity.

These studies were extended to include secondary transplantation as an additional assay for primitive stem cells. BM cells were harvested from the primary recipients of fresh and cultured fetal liver stem cells (see Figure 14. and Table 4A.) and the Ly5.2
25 positive, fetal liver-derived fraction was collected by cell-sorting. Secondary radioprotection and competitive repopulation transplants were performed. The data are presented in Table 5. The secondary recipient repopulating activities are nearly identical for the AFT024 cultured stem cells and the non-cultured controls. Lineage analysis of the Ly5.2 cells in the secondary recipients revealed similar numbers of myeloid and
30 lymphoid cells derived from both AFT024-cultured and non-cultured stem cells. Some level of secondary reconstituting cell activity was observed.

Additional experiments were also performed to determine where the levels of stem cell activity present in long-term AFT024 cocultures were compared to those present

in short-term cytokine stimulated cultures or in short-term AFT024-supported cultures (Figure 15). Purified fetal liver cells were seeded onto an AFT024 monolayer and maintained in Dexter-LTC conditions for 5 weeks. Simultaneously, the same numbers of purified cells were cultured for 5 days with; (1) different cytokine combinations or (2) on AFT024. The transplantable activity in the cultured cells was then assayed by competitive repopulation. Each mouse received the cultured equivalent of 600 stem cells together with 4×10^5 Ly5.1 competitor BM cells. It is evident from the data that the levels of *in vivo* repopulating activity present in the long-term AFT024-supported cultures are much greater than those remaining after a short-term cytokine-supported culture period. Of interest also is that short-term AFT024 stem cell cocultures do not maintain significant *in vivo* reconstituting activity. In fact, these levels of stem cell activity are identical to the levels seen in the cytokine-supported cultures.

In vitro stem/progenitor populations are expanded by AFT024. AFT024/stem cell cocultures have vigorous hematopoiesis throughout the entire *in vitro* culture period. This is reflected in the large numbers of relatively mature hematopoietic cells which are produced throughout the culture period. In addition, CA colonies are observed throughout the culture period. Figure 16 shows a time course of CA appearance with purified fetal liver stem cells (3 separate experiments). After 28 days in culture, approximately one in every twenty input stem cells is capable of proliferating into a CA. In addition, CA appearance over time follows a biphasic distribution, with many CA observed early in the culture period. In order to enumerate the various classes of stem/progenitor cells present in AFT024 cocultures, we performed a series of *in vitro* replating experiments. These included the quantitation of: (1.) progenitor cells capable of colony-formation in cytokine-supplemented semisolid cultures (CFU assay), (2.) progenitor cells capable of initiating secondary CA in limiting-dilution AFT024 cultures and (3.) progenitor cells which can initiate B-lymphopoiesis in LD-WW cultures. In all of these experiments the primary cultures were initiated with purified fetal liver cells. All data presented below are normalized to an initial input of 10^3 purified cells (CFU assay) or the actual number of initial input stem cells (limiting-dilution assays).

Shown in Figure 17 are the numbers and types of cytokine responsive CFU progenitors present at various times in the AFT024-supported cocultures. Production of CFU is evident at all time points. However, the content is especially high after four weeks, representing a 5-7 fold increase/expansion when compared to the content in the

freshly purified populations. The content of more primitive progenitors (CFU-HPP-Mix) is increased by 12-fold. These HPP-Mix colonies often reach a size of 2 mm in 8 days and contain large numbers of erythroid bursts and megakaryocytes. Interestingly, there does not appear to be a correlation between CA number and CFU content at different culture times. This is most apparent at day 6, when CA numbers are at their peak but the progenitor content is similar to that observed in non-cultured stem cells. Furthermore, there is no correlation between CFU content and the absolute numbers of maturing hematopoietic cells present in a given culture.

Next, the content of primitive B-lymphoid progenitors present in the AFT024/stem cell cultures was determined. This was accomplished by plating cells from the four-week AFT024 cocultures into LD-WW assays over the 2018 stromal cell line. Two experiments with freshly purified stem cells and AFT024-cultured stem cells showed that the frequency of pro-B cell progenitors is expanded 10-fold in AFT024 cultures compared to the frequency observed in the freshly purified input population (day 0 frequency 1 in 11.0, $r^2 = 0.98$; day 28 AFT024-cultured frequency 1 in 1.1, $r^2 = 0.97$).

In order to measure the content or frequency of progenitor cells capable of initiating secondary CA, 4 separate, four-week AFT024/stem cell cocultures were replated in limiting-dilution onto fresh AFT024 monolayers. CAs were scored after one week. The data are presented in Figure 18A. Large numbers of secondary CAs were observed. When normalized to the stem cell numbers used to initiate the primary cultures (stem cell equivalents, see Methods), the frequency of these progenitors is 1 in 3 to 4. Figure 18B. shows data from one of the 4 experiments presented in Figure 18A., where the quantitation of secondary CAs was extended for 4 more weeks. The frequency of CA decreases slowly over time (1 in 19 after an additional 4 weeks), approximating the frequency seen in the primary cultures at four weeks. In summary, our *in vitro* replating assays collectively demonstrate a significant expansion of primitive progenitor populations in 4 week AFT024 cultures. In these same cultures there is no decrease in the levels of transplantable stem cell activity present in the total hematopoietic cell population.

Discussion

In this example, it was demonstrated, that the AFT024 stromal cell line can maintain quantitative levels of *in vivo* repopulating stem cells for at least 7 weeks of *in vitro* culture. Highly enriched stem cell populations in low numbers (100 cells) were

used and cell activity was measured in a stringent, competitive repopulation assay system. The cultured stem cell activity satisfies all *in vivo* criteria normally ascribed to the most primitive stem cell compartment; (1) long-term engraftment ability, (2) multilineage potential and (3) the ability to repopulate secondary recipients. In addition, the studies with the low culture initiating stem cell numbers for both BM and fetal liver, imply that AFT024 stromal cells exert their supportive effects in a direct manner. These studies represent a clear example of an *in vitro* system capable of directly supporting the most primitive stem cell compartment.

Other data revealed that stromal cell lines isolated from a single tissue source are heterogeneous with respect to their abilities in maintaining long-term repopulating stem cells. (Wineman, et al., 1996). It was speculated that the rare cell lines which were effective in supporting *in vivo* reconstituting stem cells may represent immortalized components of *in vivo* stem cell niches. However, the data argue for the necessity of using purified stem cell populations in order to support such a hypothesis. Specifically, the 2018 cell line maintained transiently reconstituting activity present in unfractionated BM. However, 2018 fails to maintain measurable repopulating activity when cultured for 4-6 weeks with highly purified BM or fetal liver stem cells. Similarly, in experiments using purified cells, there was a failure to show robust levels of reconstituting stem cell activity in cultures supported by CFC034, the most effective cell line in the whole BM studies (Wineman, et al., 1996). The 2012 cell line which was reported (Wineman, et al., 1996) to be effective in maintaining long-term repopulating whole BM derived stem cells is only partially effective in the present studies. Moreover, only some subclones of 2012 display such activity (in spite of identical proviral integration positions in all subclones). Recently, studies have shown that the S17 cell line which consistently supports the stem cell activity present in whole BM, (Wineman, et al., 1993) is not similarly effective in the maintenance of purified BM stem cells. (Szilvassy, et al., 1996). Taken together with these current data, the previously observed stem cell supporting stromal cell activities may reflect the action(s) of indirect mechanisms and therefore do not permit the identification of cellular stem cell niche components. One previous study has shown that the Sys1 stromal cell line can maintain high levels of transplantable activity present in purified BM. (Szilvassy, et al., 1996). The competitor cells in that study were compromised by prior serial transplantation. Moreover, the culture period was extended for only two weeks and effective maintenance required the addition of exogenous

leukemia inhibitory factor. In contrast, AFT024 is a cell line that provides a direct-acting long-term stem cell supporting environment without the addition of exogenous factors.

In addition to recovering net input levels of transplantable activity from AFT024 supported cultures, significantly expanded populations of primitive progenitor cells were also obtained. CFU-HPP-Mix progenitors are expanded by 12-fold after 4 weeks of culture and the numbers of stromal-dependent pro-B lymphoid progenitors are similarly amplified. This suggests that the AFT024-mediated process of stem cell maintenance is in reality a dynamic phenomenon. Specifically, during the first portion of the culture period, the majority of transplantable stem cell activity may be lost, through differentiation or cell death. The remaining primitive stem cells may expand to yield input levels of transplantable activity as well as increases in the numbers of more committed progenitors. One hypothesis is that short-term AFT024 supported cultures should contain reduced levels of transplantable stem cell activity. Figure 17 support this hypothesis. The standard Dexter-type media used in parallel long-term AFT024 cocultures was not used in these short-term cultures. However, in another short-term experiment, utilizing Dexter-LTC media, a similarly dramatic reduction in stem cell activity was observed after 4 days of culture on AFT024.⁶³ These observations are intriguing because they suggest that the AFT024 cell line is able to facilitate some degree of *ex vivo* transplantable stem cell proliferation and expansion. Indeed, in other studies it was shown that AFT024 can support colony formation initiated by single purified stem cells with B and T-lymphoid, myeloid and erythroid potentials. Moreover, the data suggest that is possible to efficiently introduce retroviral markers into transplantable stem cells at various times during AFT024 cocultures. Extension of such marking experiments and an analysis of proviral integration patterns will be necessary to rigorously ascertain if self-renewal replication is occurring during these coculture periods.

The ability of AFT024 to maintain the most primitive stem cell compartment while generating and expanding at least some less primitive members of the stem/progenitor cell hierarchy raises interesting issues regarding the nature of stem cell niches. The present invention suggests that microenvironmental niche models which postulate distinct cellular entities responsible for stem cell self-renewal and other cellular entities which support the generation of committed progenitor cells may be overly simplified. (Uchida, et al., 1993). Quite clearly a single microenvironmental cell type represented by AFT024 is sufficient for keeping stem cells in an undifferentiated state as

well as allowing commitment and progenitor expansion to take place. A hallmark feature of a stem cell niche is the ability to facilitate the generation of the entire stem/progenitor cell hierarchy from very primitive cells. Therefore, the main functional role of such niches may be to provide an environment which permits the production of the correct numerical balance of more and less primitive stem/progenitor cell entities. This model contains several testable hypotheses. The most important is that *in vitro* stem cell maintenance should not be interpreted, literally, as the maintenance of quiescent cells but rather as a phenomenon which results from a balance of self-renewal and commitment decisions which occur during stem cell division.

A cytokine cocktail of IL-6, SL, and FL is not effective in maintaining fetal liver stem cell activity. It was shown that RNA transcripts for these and 10 other cytokines are present in AFT024, but they are also detected at similar levels in non-supporting lines such as 2018. (Wineman, et al., 1996). These observations suggest the existence of novel AFT024-derived molecules which may act on stem cells. Indeed, using a subtractive hybridization molecular cloning strategy, a number of candidate molecules have been identified. Two of these molecules contain EGF-like repeat motifs that are most closely related to those found in the Notch/Notch-ligand family. Interestingly, one of these molecules appears to have activity on primitive stem cell populations. (Moore, et al., 1997). (See, Examples presented herein above.

Table 4. Multilineage stem cell activity, high CRU levels maintained in AFT024 cultures
A. With 1000 purified fetal liver cells

Cells	Week	Lineage Contribution, % Ly 5.2 Cells				Total	
		CD4	CD8	B220	Myeloid	% Ly 5.2	CRU/10 ⁵
Control	5	3.3±1.5	4.4±1.7	48±5.1	32±6.0	28±1.3	386±24
	12	27±10	24±8.7	63±8.9	43±9.2	44±3.2	800±102
	24	42±6.9	34±4.1	71±4.4	62±1.4	48±4.8	984±171
AFT024	5	7.7±6.2	6.0±3.5	58±6.6	36±4.7	39±2.1	641±54
	12	44±7.1	38±6.4	70±5.6	53±3.6	48±1.4	942±51
	24	56±3.8	49±3.1	72±2.4	64±3.7	56±1.9	1270±96
2012	5	0.8±0.5	1.4±0.3	6.8±3.6	14±11	7.4±2.3	52±8.5
	12	9.1±8.4	10±6.9	14±9.3	23±20	14±4.5	194±68
	24	17±3.8	18±2.0	23±5.9	32±6.4	21±4.4	196±71

B. With 100 purified fetal liver cells

Cells	Week	Lineage Contribution, % Ly5.2 Cells			Total	
		Granulocytes	B-cells	T-Cells	% Ly5.2	CRU/10 ⁵
AFT024	15	33±4.6	41±6.0	25±5.3	28±4.6	1888±315
	26	17±4.4	18±4.8	18±5.9	24±5.9	1825±571
	46	22±5.7	19±6.6	15±6.7	20±6.5	1595±628
2012	15	32±7.8	28±7.2	20±5.2	24±6.6	1390±382
	26	10±2.8	9.7±3.2	9.7±3.2	14±3.9	699±176
	46	11±2.3	6.7±1.6	8.9±3.2	9.0±2.0	409± 80

C. With 100 purified bone marrow cells

Cells	No. of mice	Lineage Contribution, % Ly 5.1 Cells				Total	
		CD4	CD8	B220	Myeloid	% Ly 5.1	CRU/10 ⁵
Control	8	38±7.3	30±5.8	43±6.7	31±4.8	32±6.5	583±172
AFT024	7	20±9.8	18±8.8	20±8.7	24±11	22±10	490±296

5 A. AA4.1⁺ day 14 fetal liver cells (Ly 5.2) were further purified for a lin^{-lo}, Sca-1⁺, ckit⁺ stem cell surface phenotype. Fresh purified control cells, 10³, were transplanted with 10⁶ Ly5.1 competitor marrow (n=6 mice). From the same purification, 10⁴ cells were cocultured with stromal cell lines for 4 weeks. Subsequently, 10% of each culture was transplanted per mouse (n=8 mice/stroma) together with 10⁶ competitor BM cells. The contribution to each lineage in peripheral blood is expressed as the percent of the total specific lineage population that was Ly5.2⁺. CRU/10⁵, relative enrichment of competitive

10 repopulating units. Data are presented ±SEM.

B. The lineage and CRU content of low numbers of enriched fetal liver stem cells maintained on AFT024 and 2012 were determined. 500 stem cells were maintained in Dexter-LTC for 4-7 weeks over irradiated monolayers. 20% of each culture was used to transplant groups of 4 mice (i.e. each mouse received the

15 equivalent of 100 stem cells that initially seeded the cultures) combined with 4X10⁵ competitor BM cells. Data are presented as ±SEM.

C. Ly 5.1 BM cells with a lin^{-lo}, Sca-1⁺, and c-kit⁺ cell surface phenotype were purified. 100 fresh cells per mouse (Control) were transplanted with 10⁵ Ly 5.2 competitor BM cells. One thousand of the same

20 purified cells were cocultured with stromal cell lines for 6 weeks. The cultures were then harvested and 10% of each culture was transplanted per mouse with competitor. Data are from peripheral blood samples taken 4 months after transplant and are presented ±SEM.

Table 5. LTRSC maintained on AFT024 are able to repopulate secondary recipients at levels comparable to non-cultured stem cells.

Group	Weeks	% Ly 5.2 ⁺ peripheral blood cells	
		Radioprotection	Comp. Repopulation
Control	6	21 (1)	2.6±0.6 (4)
	22	54 (1)	5.6±1.6 (4)
AFT024	6	13±0.2 (4)	1.3±0.9 (8)
	22	44±15.1 (3)	4.3±0.9 (8)
2012	6	6.7±2.9 (4)	ND
	22	14±2.2 (4)	ND

The retransplantation potential of LTRSC in primary recipients of stromal cell cultured stem cells was studied in secondary recipients. 60 weeks after transplant, primary mice (see Figure 14, Table 4A.) were sacrificed, BM harvested and stained with antibody to Ly5.2. Ly5.2⁺ cells were collected by cell sorting and used to transplant secondary recipients (congenic Ly5.1 mice). Control and AFT024 groups were transplanted with 1.5×10^6 Ly5.2 cells/mouse for radioprotection (4 mice/group were transplanted) and 7.5×10^5 Ly5.2 cells + 7.5×10^5 Ly5.1 cells for competitive repopulation (4 mice for the Control group and 8 mice for the AFT024 group). 2012 mice were transplanted with 3×10^5 Ly5.2 cells/mouse (4 mice). Weeks are the times after transplant that the mice were analyzed. (n), number of mice surviving/group; ND, not done. Data are presented ±SEM.

15

EXAMPLE 4: Hematopoietic activity of a stromal cell transmembrane protein containing epidermal growth factor-like repeat motifs

Primitive hematopoietic stem cells are closely associated with discrete *in vivo* microenvironments. These “niches” are thought to provide the molecular signals that mediate stem cell differentiation and self renewal. The fetal liver microenvironment was dissected into distinct cellular components by establishing an extensive panel of stromal cell lines. One particular cell line maintains repopulating stem cells for prolonged *in vitro* culture periods. A subtraction cloning strategy has yielded a cDNA which encodes a cell surface glycoprotein with a restricted pattern of expression among stromal cell lines. This

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molecule, previously identified as delta-like/pre-adipocyte factor-1, contains epidermal growth factor-like repeats which are related to those in the notch/delta/serrate family of proteins. The potential role of this molecule in hematopoietic stem/progenitor cell regulation was investigated. The delta-like protein displays activity on purified stem cells by promoting the formation of "cobblestone areas" of proliferation. These cobblestone areas contain both primitive high-proliferative potential progenitors and *in vivo* repopulating stem cells.

Introduction

The positive and negative regulatory mechanisms that govern the proliferation, self-renewal and differentiation of primitive hematopoietic stem cells are complex and poorly understood (Ogawa, M. (1993) *Blood* 81, 2844-2853). Numerous cytokines have been identified that, when used *in vitro* appear to act directly on purified stem cells by promoting proliferation and differentiation. However, attempts to demonstrate the *in vitro* maintenance and/or expansion of transplantable pluripotent stem cells using defined cytokine combinations have been largely unsuccessful (Knobel, K. M., et al., 1994; Peters, S. O., et al., 1995). Moreover, it is unclear to what extent any currently identified cytokines reflect mechanisms that are responsible for regulating normal, *in vivo*, stem cell behaviors. It is widely accepted that *in vivo*, stem cells are intimately associated with discrete microenvironmental "niches" (Wolf, N. S. 1979). Such niches are likely sources for the molecular signals which collectively mediate the differentiation and self-renewal of stem cells. Indeed, it has long been possible to demonstrate that preestablished stromal cell monolayers derived from hematopoietic tissues can support long-term hematopoiesis *in vitro* (Dexter, T. M., Allen, T. D. & Lajtha, L. G. 1977). The long-term nature of these cultures, together with the continuous production of committed progenitor cells suggest that both self-renewal and commitment decisions can occur *in vitro*. At the cellular level, the hematopoietic microenvironment consists of numerous distinct cell types. Previous studies have shown that this cellular heterogeneity reflects a similarly broad heterogeneity in terms of hematopoietic supportive abilities (Deryugina, E. I., et al., 1994). Some cloned stromal cell lines can support stem cell activity *in vitro*, while others are ineffective. Similarly, distinct stromal cell types appear to influence the outcomes of stem cell differentiation processes (Friedrich, C., et al., 1996). Recent studies have shown that stromal cell lines that efficiently maintain long-term transplantable stem cells *in vitro* for prolonged intervals represent a small fraction of the total stromal cell population (Wineman, J., et al., 1996). A fetal liver stromal cell line, AFT024 was identified which maintains high levels of

transplantable multilineage stem cell activity for extended *in vitro* culture periods (Moore, K. A., Ema, H. & Lemischka, I. R. (1997) (See, Example 3). The stem cells used to initiate these cultures are highly purified. It is, therefore, likely that in this system the mechanisms that mediate stem cell maintenance do so by acting directly on primitive stem cells. A
5 different fetal liver-derived cell line, 2018, fails to maintain long-term repopulating stem cell activity. A PCR-based RNA expression analysis of 13 cytokines reveals qualitatively identical expression patterns in AFT024 and 2018. It was therefore, hypothesized that the hematopoietic supportive ability of AFT024 is, at least in part, mediated by novel gene products not expressed in 2018.

10 Using a subtractive hybridization approach a number of cDNA clones were identified which were specifically expressed in AFT024 but not in 2018. The sequence of one cDNA was identical to a molecule that encodes a transmembrane protein that contains six epidermal growth factor (EGF) repeat motifs. The EGF-like repeat sequences of this molecule, variously known as delta-like (dlk) (Laborda, J., et al., 1993), preadipocyte factor-1 (Smas,
15 C. M. & Sul, H. S, 1993), and stromal cell protein-1 (Genbank, D16847), are most closely related to those present in the notch/delta/serrate family of signaling proteins. In *Drosophila* and *Caenorhabditis*, these molecules are required for correct cell-fate specification decisions in a variety of tissues (Rebay, I., et al., 1991). Vertebrate homologs of the notch/delta/serrate family have been identified (Ellisen, L. W., et al., 1991; Bettenhausen, B.,
20 et al., 1995; Lindsell, C. E., et al., 1995). While the exact functional relationship of dlk to the activities of this family of molecules is unclear, in one *in vitro* study, it has been shown to block adipocyte differentiation (Smas, C. M. & Sul, H. S, 1993). No studies have been reported that demonstrate a hematopoietic function for dlk. Expression analyses and these observations show a limited temporal pattern of dlk expression during murine fetal
25 development which coincides with the time period of hematopoietic stem cell expansion (Smas, C. M. & Sul, H. S, 1993).

Functional studies were undertaken to determine if dlk can act as a hematopoietic regulator. This molecule affects highly enriched stem cell populations by promoting "cobblestone area" (CSA) colony formation in dexter-type stromal cocultures. These CSA
30 colonies contain an expanded population of primitive, high proliferative potential myeloid-erythroid progenitors. These cultures also contain stem cells capable of *in vivo* engraftment at levels equivalent to those present in parallel AFT024 supported cultures. It is proposed that dlk represents one molecular component responsible for the hematopoietic supportive

ability of AFT024. As such, dlk may define a novel molecular pathway of stem cell regulation by the microenvironment.

Materials and Methods

Cell Lines and Culture. The fetal liver stromal cell lines used in this study were
5 derived as previously described (Wineman, J., et al., 1996). Cells were routinely cultured in
DMEM containing 10% fetal bovine serum (FBS) and 50 uM b-mercaptoethanol (2-ME),
and maintained at 31⁰-33⁰C, 5% CO₂. For long-term cocultures with hematopoietic stem
cells, confluent monolayers were irradiated (20 Gy), placed in modified Dexter media
(DMEM, 10% FBS, 10% horse serum, 50 uM 2-ME, 0.1 uM hydrocortisone) and
10 maintained at 37⁰ C, 5% CO₂ with weekly media changes. NIH3T3 cells were obtained from
ATCC.

dlk Expression Analysis. Total RNAs from stromal cell lines were poly A⁺ selected,
Northern blotted, and hybridized to ³²P-labeled probes according to standard protocols
(Sambrook, J., Fritsch, E. F. & Maniatis, T, 1989). A 600 bp dlk-cDNA clone from the
15 AFT024 subtracted library was used as a probe. cDNA templates for RT-PCR were
prepared according to manufacturers' protocols (GIBCO/BRL). Oligonucleotide primers
were: sense 5'- GACCCAGGCTGCCCC-3' (SEQ.ID.No.:85) and antisense 5'-
GGTACTCTTGTTGAG-3' (SEQ.ID.No.:86). For analysis of dlk expression at the protein
level, antisera specific for dlk was generated by immunizing rabbits with a Flag-dlk fusion
20 protein (described below). Resultant antibodies were purified by affinity chromatography.
Cell surface expression of dlk in stromal cell lines was accomplished by flow cytometry.
Cells were incubated with dlk antibody and a similarly prepared irrelevant control antibody.
Specific labeling was developed by donkey anti-rabbit-fluorescein isothiocyanate (Jackson
ImmunoResearch). Stained cells were analyzed on a Becton Dickinson FACScan using Cell
25 Quest software.

dlk Fusion Protein Preparation. The expression plasmid pCD4-Ig contains cDNA for
the extracellular domain of human CD4 fused to genomic sequences of the human
immunoglobulin heavy chain (Zettlmeissl, G., et al., (1990). cDNA for CH2-CH3 of human
IgG₁ (Goodwin, R. G., et al., 1990) was cloned into *EcoRI* and *NotI* sites of pcDNA3
30 (Invitrogen) to give the plasmid KB52.3.2. cDNA encoding the extracellular domain of dlk
was obtained by RT-PCR with primers BP 151 and BP 152 using total RNA from NIH 3T3
cells as template. The resulting PCR fragment was cloned into KB52.3.2 via *HindIII* and

*Eco*RI sites to obtain the soluble dlk-Ig expression plasmid. pdlk-Ig or pCD4-Ig were transfected into NIH3T3 cells together with pSVNeo and stable clones were isolated. Soluble CD4-Ig and dlk-Ig fusion proteins were harvested and then purified by affinity chromatography on HiTrap Protein G-sepharose (Pharmacia). Primers: sense BP 151,
 5 5'GAGGGTACCAAGCTTCGTGGTCCGCAACCAGAAG-3' (SEQ.ID.No.: 87);
 anti-sense BP 152, 5'-CTCAGATCTGAATTCGGCCTGTCCCTCGGTGAGGAG-3' (SEQ.ID.No.: 88).

Flag-dlk fusion protein was used to immunize rabbits for the production of dlk antiserum. The protein expression plasmid pcDNA3-Flag is a modification of the plasmid
 10 pcDNA3 (Invitrogen) and contains the coding region for the Flag peptide (DYKDDDDKI) (Hopp, T. P., et al., 1988) as well as a *Bgl*II restriction site. A cDNA fragment encoding the extracellular domain of dlk was obtained by RT-PCR using RNA from NIH3T3 cells. Primers: sense BP 155,
 5'-GACAAGATCTCAGCTGAATAGCGACCCACCCTGTG-3' (SEQ.ID.No.: 89);
 15 antisense BP 154,
 5'-GCATCTAGAGCGGCCGCTCAGGCCTGTCCCTCGGTGAGGAG-3' (SEQ.ID.No.: 90). The PCR fragment was ligated into pcDNA3-Flag to yield pFlag-dlk. pFlag-dlk was transfected into cos cells. Purification of the Flag-dlk protein from cos-conditioned media was performed according to manufacturer's directions using the Flag monoclonal antibody,
 20 M1, immobilized on agarose (International Biotechnologies).

Plasmid Constructs and Stable Transfection. Full-length murine dlk cDNA was obtained by RT-PCR with primers BP 151 (see above) and antisense BP 200: 5'GCATCTAGAGCGGCCGCGAACGCTGCTTAGATCTCCT-3' (SEQ.ID.No.:91), using total RNA from NIH3T3 cells as template. The product was subcloned into the vector
 25 pCRII (Invitrogen) and then cloned into a retroviral expression vector (Kitamura, T., et al., 1995), (G. Nolan, Stanford University), via the primer-encoded *Hind*III and *Not*I sites. Supercoiled plasmid was transfected into BFC012 stromal cells together with the pZeo (Invitrogen) selectable marker and selected in 50 ug/ml Zeocin (Invitrogen). BFC012 cells also were transfected with pZeo alone and selected as above. Clones from both selected
 30 populations were isolated and all remaining colonies (100-200 per dish) were pooled and expanded as populations.

Hematopoietic Stem Cells and *In Vitro* Hematopoietic Assays. Hematopoietic stem cell populations were derived from wild type, Ly5.2-C57Bl/6J (Jackson Laboratories), day

14 fetal liver, enriched for the AA4.1⁺, Sca-1⁺, c-kit⁺, and lin^{lo/-} phenotype, by immunopanning and fluorescence-activated cell sorting as described (Jordan, C. T., et al., 1995). Adult bone marrow (BM) was used directly after density centrifugation and immunomagnetic bead depletion or was further enriched for Sca-1⁺, c-kit⁺, lin^{lo/-} cells by
5 flow cytometry as described (Okada, S., et al., 1992). Cell sorting and data analysis was accomplished with a Becton Dickinson FACS Vantage using Cell Quest software. Stromal cell/stem cell cocultures were initiated in 12-well trays with 300-1,000 enriched stem cells per well. Cobblestone areas were quantitated by inverted-phase microscopy as described (Ploemacher, R. E., et al., 1991). Clonogenic progenitor assays were performed with either
10 freshly purified stem cells or cells harvested from the stromal cocultures. These were cultured in cytokine-containing semisolid media according to the manufacturer's recommendations (Stem Cell Technologies, Vancouver, BC). Soluble dlk and control fusion proteins were added to semisolid progenitor assays at concentrations of 0.1, 0.5 and 1.0 ug/ml and also to BFC012 stromal cocultures at concentrations of 0.1 ug/ml. Fusion protein
15 was replenished weekly in the stromal cocultures.

Competitive repopulating transplantation assay. Cultured cells were harvested, combined with fresh unfractionated BM obtained from congenic C57Bl/6 Ly5.1 mice (National Cancer Institute) and transplanted into lethally irradiated (10 Gy, split dose 3 h apart from a ¹³⁷Cs source, 1 Gy/min) Ly5.1 recipient mice. Each mouse received 2 X 10⁵
20 competitor BM cells and a fraction of the cocultured stem cells. Mice were bled by capillary puncture of the orbital venous plexus and 100 ul was collected; red blood cells were removed by NH₄Cl lysis. The nucleated cells were stained for the Ly5.2 (CD45.2) allelic marker using either fluorescein isothiocyanate-labeled directly conjugated Ly5.2 monoclonal antibody or a biotinylated form developed with streptavidin conjugated to Texas red. Cells
25 also were stained with directly conjugated antibodies to lineage markers. All antibodies and chromogens were obtained from Pharmingen. Flow cytometric analysis was done on a Becton Dickinson FACS Vantage using Cell Quest software.

Results

Genes expressed in AFT024 but not in 2018 were identified by a subtractive cloning
30 approach. Sequence analysis identified one of these AFT024-specific clones as dlk. Expression studies, (Figure 19A) show high levels of dlk in AFT024 and subclones isolated from this line, but undetectable levels in 2018 and BFC012. The latter two stromal cell lines

do not support repopulating stem cells. The PA6 stromal cell line and NIH Swiss 3T3 cells both show expression of dlk and were the cell sources for identification of SCP-1 and dlk, respectively. PA6 cells have been shown to support *in vitro* hematopoiesis and long-term, *in vivo* repopulating stem cells (Kodama, H., et al., 1992). Swiss 3T3 cells are also capable of supporting multipotent hematopoietic stem cells *in vitro*, promoting CSAs and maintaining *in vivo* spleen colony-forming units (CFU-S) (Roberts, R. A., et al., 1987). Interestingly, an additional fetal liver stromal cell line, 2012, which has some degree of stem cell supporting activity (Wineman, J., et al., 1996), and its subclones also express dlk. Furthermore, an RT-PCR analysis (40 cycles) of an additional 10 fetal liver-derived stromal cell lines and several other lines, shows detectable levels of dlk in only two additional lines (Figure 19B). These two cell lines (CFC032 and CFC008) can maintain some level of long-term transplantable stem cell activity present in whole BM (Wineman, J., et al., 1996). A correlation between a stromal cell line's ability to support stem cells and the expression of dlk was suggested. Therefore, functional studies were undertaken in order to delineate if dlk can act on or modulate hematopoietic stem cells.

Soluble dlk protein was added to progenitor cultures in semi-solid media. The soluble protein consisted of the dlk extracellular domain fused to the Fc portion of human IgG₁. The stem cell sources in these assays were highly enriched fetal liver cells (AA4.1⁺, lin^{lo/-}, Sca-1⁺, c-kit⁺). The influence of soluble dlk on hematopoietic progenitor colony-formation was assessed. As shown in Table 6, no differences were noted either in the number, sizes, or lineage compositions of colonies. Identical results were obtained at dlk concentrations ranging from 0.1 to 1.0 ug/ml. In addition, no differences were noted in similar studies using enriched BM cells (Sca-1⁺, c-kit⁺, lin^{-lo}).

Evidence for a positive effect of the dlk protein on stem/progenitor cells was observed when the soluble form was added to dexter-type cocultures. For these studies a stromal cell line (BFC012) was used that neither expresses endogenous dlk (see Figure 19) nor maintains significant *in vitro* hematopoiesis. In four experiments, two each using highly enriched adult BM and fetal liver stem cells, we monitored the appearance of CSAs over time. These colonies provide a convenient, quantitative estimate of hematopoietic activity initiated by primitive stromal dependent stem/progenitor cells. As shown in Figure 20, the addition of soluble dlk (0.1 ug/mL) results in an approximately 2-fold increase in the number of CSAs initiated by purified fetal liver or BM stem cells over a 2 week time period (P= 0.001 for dlk vs control and P= 0.01 for dlk vs no additive, Student's t-test). There was no

difference in the numbers of CSA observed in BFC012/stem cell cultures with or without control fusion protein (mean of no additive/control = 0.96 ± 0.11)

In order to assess the activity of the normal transmembrane form of dlk, a full-length dlk cDNA was transfected into BFC012 cells. Expression of the introduced dlk was demonstrated at the RNA (Northern blot) and protein levels using both Western blot and flow cytometric analyses with rabbit anti-dlk antibodies. The flow cytometry data are presented in Figure 21.

Initially, dlk-expressing transfected populations (BFC-dlk) were compared to a negative control "mock" transfected population of BFC012 cells. A 4- to 6-fold increase in the number of CSAs was observed in two separate experiments. The maintenance of CSAs was transient, lasting less than 2 weeks. No further hematopoietic activity was observed during an additional 2 weeks of culture. Once dlk-expressing clones had been identified from the transfected populations, they were studied for their ability to support CSAs in experiments designed to more precisely identify the time course of hematopoietic activity. Ficoll-separated, lineage depleted BM was used in these experiments. Five different negative control, non-dlk expressing BFC012 cell groups (parental BFC012 cells, two "mock" transfected populations, and two "mock" transfected clones) and 3 dlk-expressing BFC012 cell groups (one transfected population and two clones) were studied. The data are presented in Figure 22A. Neither the negative control BFC populations nor the "mock" transfected BFC clones supported high numbers of CSAs. In contrast, the BFC-dlk populations and the two individual dlk-expressing clones supported significantly greater numbers of CSAs at all time points studied ($P < 0.001$ days 3,4, and 5; $P < 0.01$ days 6 and 7, Student's t-test). As observed previously, all the CSAs were transient. This experiment also indicated that the dlk-promoted hematopoietic activity peaks early, at 4 days, in this culture system. Three additional experiments using purified (AA4.1⁺, lin^{lo/-}, Sca-1⁺, c-kit⁺) fetal liver stem cells were performed using two individual clones, BFC-dlk-5 and a "mock" transfected negative control BFC-Zeo-1. The results are presented in Figure 22B. There was a dramatic and significant difference in the number of CSAs observed in the BFC-dlk-5 cultures compared to the control line ($P < 0.001$, days 4, 6, and 8, Student's t-test). As before, the effect was transient and the CSA declined in number over 2 weeks. AFT024 was included as a positive control and, in each of the three experiments, verified the quality of the input purified stem cells. In the first week of culture the numbers of CSAs observed on AFT024 were similar to the numbers in the BFC-dlk5 cultures.

In order to address the "primitiveness" of the cells that give rise to the CSA observed in the BFC-dlk cocultures, a series of *in vitro* replating experiments were performed. Individual wells were harvested at various time points of coculture and the cells were plated into semisolid cytokine-containing media. The numbers and lineage compositions of the colonies were scored after 8-12 days. As shown in Figure 23A, the CSAs obtained from day 4 BFC-dlk-5 cocultures contained numerous progenitors capable of extensive proliferation and multilineage differentiation. The total number of progenitors from the dlk-expressing cultures at day 4 was significantly expanded compared to the content in the freshly purified uncultured stem cell population ($P=0.01$, Student's *t*-test). The number and lineage composition of colonies derived from parallel day 4 AFT024 cultures was nearly identical to BFC-dlk-5 derived colonies. The content of CSAs replated at day 6 from the BFC-dlk-5 cocultures was devoid of multilineage colonies, although CFU-granulocyte-macrophages were maintained at high levels; the progenitor content in the BFC-dlk-5 cocultures continued to decrease when next sampled at 10 days. In contrast, few progenitors could be demonstrated in the BFC-Zeo-1 cultures ($P=0.001$, BFC-dlk-5 vs. BFC-Zeo-1, Student's *t*-test) (Figure 23A). Taken together, the data strongly suggest that dlk acts to promote stromal-dependent colony-formation by primitive cells capable of yielding large numbers of committed progenitors, including those endowed with a high proliferative capacity and multilineage differentiation potential. The lack of CSAs and significant progenitor maintenance in the BFC-Zeo-1 cultures argues that expression of dlk in the transfected BFC012 cells is responsible for both their ability to support CSAs and to generate/maintain primitive progenitors.

In order to determine if the CSA-containing cultures supported by BFC-dlk-5 contained stem cells capable of *in vivo* engraftment, portions of the same day 4 cocultures that were plated into progenitor assays also were used to transplant mice in competitive repopulation assays. Shown in Figure 23B are the results from two independent experiments analyzed at 10 weeks after transplant. The same BFC-dlk-5 cultures that contain CSAs and primitive CFU-high proliferative potential (HPP)-Mix progenitors also contain repopulating stem cells at levels equal to those maintained in parallel AFT024 cocultures. In addition, a significant difference exists in the levels of repopulating stem cells derived from dlk-expressing cocultures compared to non-dlk expressing BFC012 cells ($P=0.05$, Student's *t*-test). Multi-color flow cytometric analyses also demonstrated that both myeloid and lymphoid Ly5.2 cells are present in these animals. A subsequent analysis of these animals at

22 weeks demonstrated lower levels of repopulation with Ly5.2 cells derived from the AFT024 and BFC-dlk-5 supported cultures (data not shown). Most significantly, no repopulation was observed at any time point in mice that received cells cocultured on the non-dlk-expressing monolayers (Figure 23B).

5 Discussion

As part of an ongoing effort to understand the biology of the hematopoietic microenvironment, a panel of stromal cell lines from midgestation fetal liver was established and characterized. Among these cell lines, the AFT024 line has the ability to maintain nearly quantitative levels of transplantable stem cell activity for extended *in vitro* time periods (Moore, K. A., Ema, H. & Lemischka, I. R., 1997). Because these cultures are initiated with highly purified stem cell populations it is likely that the AFT024-derived molecular mechanisms responsible for this ability act directly on the stem cell population. Other stromal lines that fail to maintain stem cell activity were also identified. These observations facilitated a subtractive hybridization approach aimed at identifying potential candidate molecules whose collective actions may be responsible for the AFT024 stem cell maintenance activity. This effort has identified dlk, a transmembrane molecule containing six EGF-like repeat motifs. Although lacking the DSL motif indicative of the notch ligands delta and serrate (Tax, F. E., Yeagers, J. J. & Thomas, J. H., 1994), dlk is most closely homologous to delta/notch/serrate when compared to other EGF-like repeat containing molecules (Laborda, J., et al., 1993; Smas, C. M. & Sul, H. S., 1993). The predominant role of these types of molecules in cell growth and differentiation led us to investigate the potential role of dlk in hematopoiesis. Constitutive expression of translocated human notch (Tan-1) is found in a T-cell leukemia (Ellisen, L. W., 1991). Moreover the expression of Tan-1 in primitive human stem cells has been demonstrated (Milner, L. A., et al., 1994). Nevertheless, a functional role in hematopoiesis for the notch ligands Jagged (Lindsell, C. E., 1995) and Delta-like-1 (Bettenhausen, B., et al., 1995) has not been described. dlk expression is highly restricted in a panel of stromal cell lines. Two lines, AFT024 and 2012, which maintain repopulating stem cell activity *in vitro*, express dlk, whereas two non-supportive cell lines, 2018 and BFC012, do not. Interestingly, the S17 stromal cell line which is considered to be a potent stem cell supporter (Wineman, J. P., et al., 1993) does not express detectable levels of dlk. The S17 cell line was derived from adult BM (Collins, L. S. & Dorshkind, K., 1987)). The other lines described are all derived from fetal sources (AFT024, 2012, and NIH 3T3 cells) or from newborn calvaria (PA6 cells). It is therefore

possible that dlk acts in a developmentally regulated fashion. An extensive analysis of dlk expression in adult BM stroma is currently underway. Taken together, the data suggest that, at least in fetal stromal cell types, there exists a correlation between hematopoietic supporting ability and the expression of dlk.

5 The potential activity of both soluble and transmembrane dlk protein, on highly purified stem cell populations were directly measured using *in vitro* and *in vivo* assays. Initial experiments designed to ask if dlk can enhance colony formation in cytokine-rich semisolid assay systems were negative. These results may indicate that: (i) progenitor cells capable of colony formation in semisolid assays do not respond to dlk, (ii) the
10 collection of cytokines present in the semisolid cultures may "mask" an effect(s) of added dlk or (iii) that the soluble form of dlk requires a stromal monolayer to mediate its effects. The first possibility can be addressed more extensively in delta-type assays, where stem/progenitor cells are first cultured in suspension in serum-free media containing various cytokine combinations, with and without dlk, and then replated into colony assays (Muench,
15 J. O., Firpo, M. T. & Moore, M. A., 1993). The second possibility can be addressed by more extensive studies using subsets of the cytokines present in our initial studies. These experiments are underway. As a first step to address the third possibility, we added soluble dlk to preestablished BFC012 monolayers. Using both purified BM and fetal liver stem cell populations, a significant increase in CSA colony formation was observed in the dlk
20 supplemented cultures (Figure 20). This was a surprising result, given that dlk is a transmembrane protein; however, before its cDNA cloning, a soluble form of dlk was identified as FA1 or fetal antigen 1 (Jensen, C. H., et al., 1994). A role in hematopoiesis was not indicated in these studies, but expression was detected in stroma of placental villi, in yolk sac blood islands and in fetal liver (Jensen, C. H., et al., 1994). It is of interest to
25 determine if a soluble form is produced by the stromal cell lines that express dlk. An additional explanation, for the effects observed with the soluble form added to stromal/stem cell cocultures, is that they may be facilitated by the Fc portion of the fusion protein. It is possible that Fc receptors expressed by some of the hematopoietic cells in the cultures are able to sequester and present the dlk-Fc fusion protein more effectively. This possibility can
30 be addressed by using a different type of soluble dlk protein. These studies have been initiated. Alternatively, the soluble dlk may be sequestered and thus presented by the stromal cell extracellular matrix. In order to further address the third possibility, an intact transmembrane form of dlk was introduced into the BFC012 stromal cell line. Initially, dlk

transfected BFC012 cell populations were compared to BFC012 cells transfected with the selectable marker alone. In these studies, the dlk expressing monolayers were more effective at promoting CSA colonies. As with the soluble dlk experiments, the CSAs appeared early and were transient. When cells were replated from the BFC-dlk supported cocultures onto an AFT024 monolayer a reiteration of the burst of CSAs was seen that was maintained for 3 weeks. In addition, replating of these cocultures revealed a high progenitor content (~1 in 10) that included a high percentage (43%) of multipotential colonies including HPP-Mix. In the BFC-Zeo control populations neither replatable CSA nor CFU progenitors were maintained. Further experiments with individual clones from the transfected populations confirmed and extended the results obtained with the populations, demonstrating highly significant differences in the numbers of developing CSAs (Figure 22). However, in two experiments we observed that one dlk-transfected BFC012 clone, which expresses a very high level of dlk, supported fewer CSAs than non-dlk-expressing control cells. These cultures also suggested differentiation phenomena, as indicated by the number of rapidly accumulating nonadherent cells. Experiments utilizing this cell line were not included in our analyses. It is possible that there may be a threshold level of dlk expression necessary in these cultures and when it is surpassed the cells differentiate and die rapidly in the culture milieu provided by BFC012 cells. In addition, it is possible that an aberrant form of the dlk protein is made by this line. Further studies are necessary to clarify this issue. Nevertheless, in Figure 22A, dlk-transfected BFC012 cells (one population and two clones) show a significant enhancement of CSA formation compared to controls.

The observed low level maintenance of competitive repopulating stem cells in short-term dlk-expressing cocultures is of interest even though the activity diminished over time. These studies show that the ectopic expression of a single molecule (dlk) in a previously nonsupporting stromal cell line restores or enables hematopoietic support. This is demonstrated by maintenance of three different stem/progenitor cell compartments: (i) CFU-HPP-Mix, (ii) CSA, and (iii) short-term *in vivo* repopulating stem cells. It is also of interest that both qualitatively and quantitatively similar stem/progenitor cell compartments are maintained in short-term AFT024 supported cocultures (Moore, K. A., Ema, H. & Lemischka, I. R., 1997).

Two mechanisms underlying the effects of dlk are considered. First, it may be that some level of dlk expression is sufficient to retard potent differentiation signals provided by the BFC012 cell line. Second, dlk may provide a proliferative stimulus not normally

produced by BFC012. A more direct and perhaps relevant assay will be to eliminate the expression of dlk in AFT024 cocultures, thus maintaining other components that make up the culture "milieu." Studies to evaluate potential neutralizing antibodies and various dlk antisense strategies are underway.

5 The failure of BFC-dlk-5 supported CSAs to persist for periods longer than 1 to 2 weeks also may suggest the existence of other molecules in AFT024 that facilitate hematopoiesis. In this regard, it is interesting that our subtraction screen has yielded several other clones with expression patterns very similar to dlk. Eventually, with the addition of dlk and other AFT024 specific molecules it may be possible to reconstruct a
10 supportive phenotype. This should lead towards a further understanding of the *in vivo* hematopoietic microenvironment. In summary, it is proposed that dlk represents one molecular component responsible for the hematopoietic supportive activities of the AFT024 cell line. As such, dlk may define a novel molecular pathway of stem cell regulation by the hematopoietic microenvironment.

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The following is a list of documents related to the above disclosure and particularly to
20 the experimental procedures and discussions. The documents should be considered as incorporated by reference in their entirety.

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10 This invention may be embodied in other forms or carried out in other ways without departing from the spirit or essential characteristics thereof. The present disclosure is therefore to be considered as in all respects illustrative and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

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<213> Mus abbotti

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<213> Mus musculus

<400> 17

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<210> 22
 <211> 731
 <212> DNA
 <213> Mus musculus

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 gtgncttaca ccaatcgata catcattttc caacgccata cnetgaacca gccatgttat 660
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<210> 23
 <211> 400
 <212> DNA
 <213> Mus musculus

<400> 23
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<210> 24
 <211> 2649
 <212> DNA
 <213> Mus musculus

<400> 24
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cagctatgac catgatt. ccaagctcta atacgactca ctatagggaa ag ggtacg 180
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caccacctg tctttathtt attaaaactc cattttccga aaaaaaaaaa aaaaaaaaaa 2640
aaaaaaaaa 2649

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<210> 25
 <211> 499
 <212> DNA
 <213> Mus musculus

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<400> 25
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aaagacagag ggatcttggg ccatgagtca cagcaaggaa agcctcccac caaacatctg 180
cactggactc ctacaggaac aagaaatgaa ctatcggttt cagcccctga gacttgggct 240
taacatgatt ttcacattgt ctaccctacc taatagagca gagatgtaaa tattattctt 300
atthtagagg tctgatgcct cagctgcaat gggtgagaac tactcctcat ttattctctt 360
ccaaggcaat aaaagagaat ggaccaaaga cagtctgtca tcacatctag tcaaaagagc 420
taatgtcgca gtacaactct tcaaaagaaa aagaaaaaac aagaaaaaag taataaacag 480
atgtgttctg cttgaaaaa 499

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<210> 26
 <211> 686
 <212> DNA
 <213> Mus musculus

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<400> 26

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ggagagctca taatcgtaga ggacataatg atgggtgaaga ttctttgact catacagatg 180
tcagctcttt ccagacaatg gaccaaattg tgtctaatat gagaaactat atgcagaaat 240
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cagttatgac ttattccaaa ataggagatg aaccgccaaa ggtttttcat gcctcaactc 360
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gtgggactag aaaaaatggc tattggtcnt catatccatg aaccgagctc ntgttcntta 480
ataaagtcen nngancnag aaagactggg agatgaaaaa aggtccaccc aggaanttca 540
tcccttatga aatgaaaatt gatgccccct gccttttgaa tgaaggaatt ggccaaattn 600
aagggttttg aaattttcaa accccggaac aaccaatcc tnggaaaacc cctnnaaatg 660
aaaaaatttt tggggccctt aaaaan

```

<210> 27

<211> 651

<212> DNA

<213> Mus musculus

<400> 27

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gctgtcagta ctgocgannt canaaatgcc tggtgtttgg ganggncaaa gaaatggnn 120
gcacagacag nnnaaaaggc cggagaagnc gnntgccnc gaaaccgaag aaccacanga 180
nccctctccc ccttcgcccc cgttccangc gaacctgacn ancaaangaa nggagangan 240
accagcana ncengcaant ctatganctc ctgactggct ccanggaaan catcccgggc 300
ngggcagaaa aaatccctgg gcnnccgaga actgccccaa gccgaccaag aactgcnnnn 360
tgaancagcn nctcnagaac tgnttgctcn nccannagca nacagaagat gaacatcgac 420
atntctgect tctcctgcat ngcngccctg ggctatggnc acnnaaaaac acggcncngg 480
gaaccggaaa anggaanaan gncnncnga tngntgantg ncgcgagac acngacttt 540
cnacnanggg ggggggnann ccccccttn nttgttcena ctgggggggg gaacnncccn 600
aaattcgngc cctnnngggc ccnagggggg ggggggncn ttttctttct n 651

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<210> 28

<211> 683

<212> DNA

<213> Mus musculus

<400> 28

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ctgaggggtgt ttcttctgc acccacagcc agggggatgc cactcctccc tccggcttga 120
cctgtttctc tgcgctacc tccctccccg tctcattccg ttgtctgtgg atggtcattg 180
cagtttaaga gcagaacaga tcttttactt tggcgcgttg aaaagctagt gtacctctc 240
tcagtgtttt ggaactccatc tctentctc cantacctg cttcttactg ataattttgc 300
tgggaattcct aacttttcaa tgacattttt tttaactact atattgattg tcttttaaaa 360
aagaaaagtg catatttata caaaatgtgt atttcttata cgcttttctt tgttatacca 420
tttctcagc ttatctcttt tatatttgta gganaaactc ccatgttatg gaatccact 480
gtatgattta taaacagaca atatgtngag tgccttttgc aanaaaaagg gtgtgtttga 540
aatcatccgg antcagccan gaaactgtct ccaaaggaaa cncnacctc tctgttccct 600
gctgttntgc tgatcatccg ccanaagggt cttccccccg aattttgggt tggttntggt 660
tnccgaaaaa ttttttccgn ttn

```

<210> 29

<211> 624

<212> DNA

<213> Mus musculus

<400> 29

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ctgttttttt ttganggttt tcaaaacatt ttgaaaagca tttacttttt tgaccacgan 180
ccatganttt tcaaaaaaat ccgggggtgt gtgggttttt ggtttttgtt ttaatttttg 240
gttgcgttgc cttttttttt taatggggtt ggcccatga aatgggtgcc cactcactt 300
ctctganatc gaacngactg tgaatccgct ctttgtcnga anctgaacaa nctgtggctt 360

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ttttccaact cegtgtg. , tttctgaatg ttatgtggta agaccccgcg gs ,tggcan 420
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cnttcanacn tccctgaaaa gttcttgaaa aaanttaatt anattgtccc nnttttactg 540
ggaaaaaatt anccntactc cnnccaattt tnttcttnen antgggctga aagaaatcct 600
tgttcccan ttttcctttg ggen 624

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<210> 30
 <211> 641
 <212> DNA
 <213> Mus abbotti

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<400> 30
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caatgaggag gaatcagatg aagtggagga taacgacaat gactctgaaa tggagaggcc 120
tgtnaataga ggangcagcc gaagtgcgag agttagctta agtgatggca gcgatagtga 180
aagcagttct gcttcttcac ccctacatca cgaacctcca ccacccttac taaaaaccaa 240
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gacactttca tatcaaccaac acaacatttg attttgatct ttgctcgtg gacaaaacca 480
cagtcctgta actacagagt tccctggaaac atctggaaca tccctgaagat ataaccactg 540
gatgcctcna gaactaatgt gtttnnnnnn nnnnnngggtt nnnnnntttg ggtgtgaatt 600
ttgttcccg ttgtttaaat gaaaaccccc cnaatgatgc n 641

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<210> 31
 <211> 400
 <212> DNA
 <213> Mus musculus

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<400> 31
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gcaggggaga cccagcggca gattctgtca cagacgaggg agaaggcgtg aggagacaaa 180
gccgtcacat ccgcgacagc ttccttcagc agcctcctcc tctccagtc agagccgacc 240
cccagacccc tgaggcatcc ctcccgctct cggaacaccc tagtcattca ttgctaacag 300
gggatcatga gggacctgt aagtagccag tacagctcct ttcttttctg gangatgccc 360
atcccacaac tggatctgtc ngagctggaa ggcctgggcc 400

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<210> 32
 <211> 400
 <212> DNA
 <213> Mus musculus

```

<400> 32
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acataaggag tgactccnaa tttacataca ctggaacgga aatgcgcaca gtccnagaaa 300
agatggatac catcagaatt gttcatatcc attctgtcat catcttgccg cgttctgaca 360
aganaaanga ccgtgttnnaa atttctccan ancanctgtc 400

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<210> 33
 <211> 400
 <212> DNA
 <213> Mus musculus

```

<400> 33
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cagaatatat agcctgaaag tagaatgtgg atctaaatac ccagaagctc ctccatcagt 240
tagatttgta acaaaaatta atatgaatgg gatcaataat tccagtggaa tgggtggatgc 300
acggagcata ccagtattag caaatggca aaattcctat agcattaaag tcataactca 360

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aganctaaga ctcttatg gtccaaagaa aatatgaanc 400

<210> 34
 <211> 400
 <212> DNA
 <213> Mus musculus

<400> 34
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 actggccttg gactgacttc ttcagatccc tcataacaac catcctgtac ctgatcacct 360
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<210> 35
 <211> 400
 <212> DNA
 <213> Mus musculus

<400> 35
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<210> 36
 <211> 400
 <212> DNA
 <213> Mus musculus

<400> 36
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<210> 37
 <211> 394
 <212> DNA
 <213> Mus musculus

<400> 37
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 gtggccgctc tgaagctgcc tctcctgnta tctannctgt tggacgttcc tngatgtgg 300
 aagcgccgtt ctgaccgggt tctccacatt gacctgcgga tgtgggtga cctcatgcta 360
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<210> 38
 <211> 315
 <212> DNA
 <213> Mus musculus

<400> 38

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nctggagatg gaggatgagg acacnnttga tgtattccag cagcagacag gaggatcanc 240
ctcccagagg agcgtccac acccaaccgt tgcctgacc tgtgctattg agcagtgacc 300
atgctnccac accca 315

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<210> 39
 <211> 400
 <212> DNA
 <213> Mus musculus

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<400> 39
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tgcagtaggg tgggtggcat ggtggggggc ggcatanca caccagtggt catcccgac 180
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<210> 40
 <211> 349
 <212> DNA
 <213> Mus musculus

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<400> 40
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tcccggtgca ctgcttcac acganaaaga caagccggtt gctgaaccaa tcccatctg 180
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ctgcgcggac tgcggcaata ncggtcatcc atcgtgttta aagttctccc canagctnac 300
agtgagagtg aagccttacn gtggcttnca ttgantgtaa aacatcact 349

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<210> 41
 <211> 397
 <212> DNA
 <213> Mus musculus

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<400> 41
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gaagggtttc gtaaacagca caaggactct gggccac 397

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<210> 42
 <211> 158
 <212> PRT
 <213> Mus musculus

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<400> 42
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Lys Gly Ser Ser Gly Ser Ser Thr Val Gln Arg Ser Lys Ser Phe Ser
             20             25             30

Leu Arg Ala Gln Val Lys Glu Thr Cys Ala Ala Cys Gln Lys Thr Val
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<212> DNA

<213> Mus musculus

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PCT/US99/19052

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<211> 778

<212> PRT

<213> Mus musculus

<400> 50

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Val Ser Leu Lys Asp Ile Thr Glu Ile Met Glu Arg His Leu Ser His
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Met Glu Arg Thr Leu Ser His Ser Gln Lys Leu Ser Asp Gly Asp Leu
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Val Arg Trp Ala Ser Gly Gly Leu Val Leu Gln Gly Ile Tyr Lys Thr
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Asn His Pro Arg Ser Leu Ile Gln Lys Arg Glu Glu Leu Leu Ser Val
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Pro Lys Gln Phe Ser Leu Ala Gly Pro Val His Gly Thr Glu Ile Lys

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Asn Thr Cys Leu Ile Asp Trp Asp Leu Gln Asn Phe Leu Ile Asn Ile
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 Val Asn Phe Cys Lys Asn Ser Pro Thr Tyr Lys Thr His Phe Ile Lys
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 Ser Gln Leu Cys Ser Leu Leu Glu Pro His Val Tyr Lys Val Thr Asn
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 Leu Lys Lys Thr His Lys Tyr Leu Met Glu Glu Ser Phe Lys Thr Glu
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 595 600 605
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 660 665 670
 Gly Gln Arg Leu Thr Leu Ile Gln Gln His Met Gly Ser Leu Leu Ser
 675 680 685
 Glu Glu Val Ala His Val Leu Thr Lys His Gly Glu His His Asp Trp
 690 695 700
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<211> 1712

WO 00/11168

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<212> DNA

<213> Mus musculus

<400> 51

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<212> PRT

<213> Mus musculus

<400> 52

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Lys Thr Tyr Leu Glu His His Met Glu Lys Phe Gly Phe Gln Ala Pro
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 Lys His Gly Gly Glu Leu Tyr Phe Ser Asp Val Tyr Ala Ser Leu Glu
 180 185 190
 Val Pro Glu Asp Ile Lys Ser His Lys Val Leu Trp Gly Glu Cys Leu
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 210 215 220
 Gly Phe Cys Pro Pro Arg Leu Val Thr Ala Asp Ile Ile Thr Val Glu
 225 230 235 240
 Asn Lys Glu Leu Glu Gly Val Leu Gly Asp Cys Arg Phe Val Ser Ala
 245 250 255
 Thr Phe Arg Leu Phe Lys Leu Pro Lys Thr Glu Pro Ala Glu Arg Cys
 260 265 270
 Arg Val Val Tyr Asn Gly Gly Ile Lys Gly His Glu Lys Glu Leu Ile
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 35 40 45
 Asp Gln Ser Ser Glu Pro His Ile Phe Val Trp Asn Thr Glu Glu Thr
 50 55 60

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Leu Thr Ile Arg Ala ro Phe Leu Ala Ala Pro Asp Ile Pro A Phe
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 Phe Pro Glu Pro Arg Gly Leu Tyr His Phe Cys Leu Tyr Trp Ser Arg
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 His Thr Gly Arg Leu His Leu Arg Tyr Gly Lys His Asp Tyr Leu Leu
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 Ser Ser Gln Ala Ser Arg Leu Leu Cys Phe Gln Lys Gln Glu Gln Ser
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 Leu Lys Gln Gly Ala Pro Leu Ile Ala Thr Ser Val Ser Ser Trp Gln
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 Phe His Asn Ala Pro His Lys Val Ser His Asn Ala Ser Val Asp Met
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 Cys Asp Leu Lys Lys Glu Leu Gln Gln Leu Ser Arg Tyr Leu Gln His
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 Pro Gln Lys Ala Ala Lys Arg Pro Thr Ala Ala Phe Ile Ser Gln Gln
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WO 00/11168

PCT/US99/19052

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 Phe Leu Met Ile Gly Ile Asp Gly Ser Glu Ala Ala Asp Gly Ser Asp
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<211> 359

<212> PRT

<213> Mus musculus

<400> 58

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```

```

Tyr Ala Gly Gly Pro Ser Gly Val Ala Arg Leu Glu Ser Trp Asp Val
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```

```

Arg Asp Gly Gly Leu Gly Pro Ala Gly Asp Arg Ser Thr Gly Pro Ser
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Arg Arg Gly Glu Arg Arg Val Ile Arg Leu Ala Asp Cys Val Ser Val
          65                      70                      75                      80

```

```

Leu Pro Ala Asp Gly Glu Ser Cys Pro Arg Asp Thr Gly Ala Phe Leu
          85                      90                      95

```

```

Ile Thr Thr Thr Glu Arg Ser His Leu Leu Ala Ala Gln His Arg Gln
          100                      105                      110

```

```

Ser Trp Val Asp Pro Ile Cys Gln Leu Ala Phe Pro Gly Thr Gly Glu
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```

```

Cys Ser Ser Gly Ser Gly Gln Ala Glu Asn Pro Lys Arg Gly Phe Val
          130                      135                      140

```

```

Pro Met Glu Glu Asn Ser Ile Tyr Ser Ser Trp Gln Glu Val Thr Glu
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Phe Pro Val Ile Val Gln Lys Thr Glu Ala Thr Ser Arg Cys Gln Leu

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 Arg Cys Asp Ser Gly Glu Gly Leu Phe Ala Phe Ser Ser Pro Arg Ala
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 Pro Asp Ile Cys Gly Val Val Ala Ala Ala Ile Ala Arg Gln Arg Glu
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 Arg Leu Pro Glu Leu Ala Met Ser Pro Pro Cys Pro Leu Pro Arg Ala
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 Pro Gly Phe Glu Leu Pro Thr Pro Arg Lys Leu Pro Leu Thr Asp Pro
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 Gly Pro Gln Ser Leu Pro Leu Leu Leu Ser Pro Asn Thr Arg Arg Thr
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 Gly Ile Arg Ser Leu Cys Val Arg Val Gln Ala Asp Gln Gln Ala His
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<212> PRT

<213> Mus musculus

<400> 60

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Ser Ala Ser Val Ser Ser Val Arg Ser Arg Ser Arg Thr Thr Ser Gly
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```

```

Thr Gly Leu Ser Ser Pro Pro Leu Ala Ala Gln Thr Val Val Pro Leu
      50             55             60

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Gln His Cys Lys Ile Pro Glu Leu Pro Val Gln Ala Ser Ile Leu Phe
      65             70             75             80

```

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Glu Leu Gln Leu Phe Phe Cys Gln Leu Ile Ala Leu Phe Val His Tyr
             85             90             95

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```

Ile Asn Ile Tyr Lys Thr Val Trp Trp Tyr Pro Pro Ser His Pro Pro
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```

```

Ser His Thr Ser Leu Asn Phe His Leu Ile Asp Phe Asn Leu Leu Met
      115            120            125

```

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Val Thr Ala Ile Val Leu Gly Arg Arg Phe Ile Gly Ser Ile Val Lys
      130            135            140

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```

Glu Ala Ser Gln Arg Gly Lys Gly Leu Pro Leu Pro Leu His Pro Ala
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```

```

Val Pro His Pro Leu His Val Leu Thr Ala Thr Gly Trp Ser Leu Cys
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Arg Ser Leu Ile His Leu Phe Arg Thr Tyr Ser Phe Leu Asn Leu Leu
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Phe Leu Cys Tyr Pro Phe Gly Met Tyr Ile Pro Phe Leu Gln Leu Asn
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```

Tyr Asp Leu Arg Lys Thr Asn Leu Phe Thr His Met Ala Ser Met Gly
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Pro Arg Glu Ala Val Ser Gly Leu Ala Arg Ser Arg Asp Tyr Phe Leu
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Thr Leu Arg Glu Thr Trp Lys Gln His Thr Arg Gln Leu Tyr Gly Pro

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Asn Glu Val Glu Phe Leu Lys Met Asp Phe Asn Trp Arg Met Lys Glu
275 280 285

Val Leu Val Ser Ser Met Leu Ser Ala Tyr Tyr Val Ala Phe Val Pro
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Val Trp Phe Val Lys Asn Thr His Tyr Tyr Asp Lys Arg Trp Ser Cys
305 310 315 320

Glu Leu Phe Leu Leu Val Ser Ile Ser Thr Ser Val Ile Leu Met Gln
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His Leu Leu Pro Ala Ser Tyr Cys Asp Leu Leu His Lys Ala Ala Ala
340 345 350

His Leu Ala Cys Trp Gln Lys Val Asp Pro Ala Leu Cys Ser Asn Val
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Leu Gln Pro Pro Trp Thr Glu Glu Cys Met Trp Pro Gln Gly Val Leu
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Val Lys His Ser Lys Asn Val Tyr Lys Ala Val Gly His Tyr Asn Val
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Ala Ser Pro Pro Met Ser Pro Thr Ser Ala Ser Ile Ser Phe Ser Ala
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 35 40 45
 Gln His Arg Cys Asn Glu Asn Gly Gly Asn Leu Ala Thr Val Lys Ser
 50 55 60
 Glu Glu Glu Ala Arg His Val Gln Gln Ala Leu Thr Gln Leu Leu Lys
 65 70 75 80
 Thr Lys Ala Pro Leu Glu Ala Lys Met Gly Lys Phe Trp Ile Gly Leu
 85 90 95
 Gln Arg Glu Lys Gly Asn Cys Thr Tyr His Asp Leu Pro Met Arg Gly
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 Phe Ser Trp Val Gly Gly Gly Glu Asp Thr Ala Tyr Ser Asn Trp Tyr
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Lys Ala Ser Lys Ser ar Cys Ile Phe Lys Arg Cys Val Ser I Ile
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 Cys Gly Cys Pro Pro Gly Trp Glu Leu Ala Pro Asn Gly Val Phe Cys
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 Ser Arg Gly Thr Val Phe Ser Glu Leu Pro Ala Arg Pro Pro Gln Lys
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Pro Leu His Ser Asn Gly Val Glu Tyr Glu Ala Gln Gly Ala Glu Lys		
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Cys Gln Pro Lys Tyr Phe Val Phe Asn Ser Arg Thr Ala Tyr Ala Ile		
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Pro Ile Leu Ala Phe Ala Phe Val Cys His Pro Glu Val Leu Pro Ile		
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Ala Tyr Ser Lys Val Tyr Thr Phe Asp Thr Ala Leu Leu Met Val Arg		
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Leu Ala Val Leu Val Ala Val Thr Leu Thr Val Pro Ile Val Leu Phe		
	420	425 430
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	435	440 445
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	450	455 460
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	485	490 495
Phe Tyr Leu Lys Leu Val Lys Lys Glu Pro Leu Arg Ser Pro Gln Lys		
	500	505 510
Ile Gly Ala Leu Val Phe Leu Val Thr Gly Ile Ile Phe Met Met Gly		
	515	520 525
Ser Met Ala Leu Ile Ile Leu Asp Trp Ile Tyr Asn Pro Pro Asn Pro		
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Asn His His
545

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<213> Mus musculus

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<213> Mus musculus

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35 40 45
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50 55 60
Leu Ser Arg Leu His Ala Asn Gln Ser Arg Glu Asp Ser Asn Ser Glu
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Pro Ser Pro Asp Pro Ala Val Arg Ile Leu Ser Pro Glu Val Arg Leu
85 90 95
Gly Ser His Gly Gln Leu Leu Leu Arg Val Asn Arg Ala Ser Leu Ser
100 105 110
Gln Gly Leu Pro Glu Ala Tyr Arg Val His Arg Ala Leu Leu Leu Leu
115 120 125
Thr Pro Thr Ala Arg Pro Trp Asp Ile Thr Arg Pro Leu Lys Arg Ala
130 135 140
Leu Ser Leu Arg Gly Pro Arg Ala Pro Ala Leu Arg Leu Arg Leu Thr

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 180 185 190
 His Pro Arg Asp Ser Cys Pro Leu Gly Pro Gly Arg Cys Cys His Leu
 195 200 205
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 210 215 220
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 225 230 235 240
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 Asp Lys Gln Ile Lys Asn Gly Glu Cys Asp Lys Ala Tyr Leu Asp Glu
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 <213> Mus musculus

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<400> 72

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<212> PRT

<213> Homo sapiens

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 35 40 45

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 50 55 60

Ser Ser Pro Leu His His Glu Pro Pro Pro Pro Leu Leu Lys Thr Asn
 65 70 75 80

Asn Asn Gln Ile Leu Glu Val Lys Ser Pro Ile Lys Gln Ser Lys Ser
 85 90 95

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35 40 45

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50 55 60

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85 90 95

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100 105 110

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115 120 125

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130 135 140

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165 170 175

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195 200 205

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Cys Gly Glu Gly Asp Lys Asp Glu Thr Gln Ser His Tyr Phe Leu Cys
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245 250 255

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 Pro His Gly Lys Asn Tyr Thr Cys Arg Cys Pro Gln Gly Tyr Gln Leu
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 Cys Trp Val Gly Tyr Glu Pro Gly Gly Pro Gly Glu Gly Ala Cys Gln
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 Ser Phe His Cys Gly Cys Leu Pro Gly Trp Val Leu Ala Pro Asn Gly
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 Thr Pro Thr Thr Ser Arg Pro Ser Leu Ser Ser Asp Ala Pro Ile Thr
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 Ser Ala Pro Leu Lys Met Leu Ala Pro Ser Gly Ser Ser Gly Val Trp
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 Arg Glu Pro Ser Ile His His Ala Thr Ala Ala Ser Gly Pro Gln Glu
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 Pro Ala Gly Gly Asp Ser Ser Val Ala Thr Gln Asn Asn Asp Gly Thr
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 Asp Gly Gln Lys Leu Leu Leu Phe Tyr Ile Leu Gly Thr Val Val Ala
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Ala	Asp	Ser	Tyr	Ser	Trp	Val	Pro	Glu	Arg	Ala	Glu	Ser	Arg	Ala	Met	
625						630					635				640	
Glu	Asn	Gln	Tyr	Ser	Pro	Thr	Pro	Gly	Thr	Asp	Cys					
				645						650						

WHAT IS CLAIMED IS:

1. An isolated nucleic acid derived from an isolated hematopoietic stem cell, the isolated nucleic acid comprising the following characteristics:
 - 5 - specifically expressed in the hematopoietic stem cell; and
 - encoding a hematopoietic stem cell – specific protein.
2. An isolated nucleic acid of claim 1, the isolated nucleic acid further comprising the following characteristic:
 - 10 - capable of hybridizing under standard conditions with a sequence selected from the group consisting of SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25, SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39, SEQ.ID.No.: 40, SEQ.ID.No.: 41, SEQ.ID.No.: 42, and SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a portion thereof.
- 25 3. The isolated nucleic acid of claim 1, the isolated nucleic acid further comprising the characteristic of encoding a protein capable of modulating hematopoietic stem cell activity.
4. The isolated nucleic acid of claim 3, wherein the activity is selected from the group consisting of hematopoietic stem cell differentiation and hematopoietic stem cell replication.
- 30

5. The isolated nucleic acid of claim 3, wherein the protein is selected from the group consisting of a growth factor, a transcription factor, a splicing factor, a capping factor, a transport protein, a translation factor, and a replication factor
- 5 6. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises the nucleotide sequence of SEQ.ID.No.: 72, an analog thereof, or a portion thereof.
7. The isolated nucleic acid of claim 1, wherein the hematopoietic stem cell is a primitive hematopoietic stem cell.
- 10 8. The isolated nucleic acid of claim 7, wherein the primitive hematopoietic stem cell is selected from the group consisting of an umbilical cord cell, a bone marrow cell and a fetal liver cell.
- 15 9. The isolated nucleic acid of claim 7, wherein the primitive hematopoietic stem cell is selected from the group consisting of a AFT024 cell, a 2012 cell and a 2018 cell.
10. A composition comprising the nucleic acid of claim 1, wherein the nucleic acid comprises one selected from the group consisting of SEQ.ID.No.: 1, SEQ.ID.No.: 2, 20 SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25, SEQ.ID.No.: 26, SEQ.ID.No.: 27, 25 SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39, SEQ.ID.No.: 40, SEQ.ID.No.: 41, SEQ.ID.No.: 42, SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, 30 SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a portion thereof.

11. The nucleic acid of claim 1, wherein the nucleic acid is selected from the group consisting of DNA, RNA and cDNA.
12. A vector comprising the nucleic acid of claim 1.
- 5 13. The vector of claim 12, wherein the vector comprises viral or plasmid DNA.
14. An expression vector comprising the nucleic acid of claim 1 and a regulatory element.
- 10 15. A host vector system which comprises the expression vector of claim 12 in a suitable host.
16. The host vector system of claim 15, wherein the suitable host is selected from the group consisting of a bacterial cell, a eukaryotic cell, a mammalian cell and an insect cell.
- 15 17. An isolated hematopoietic stem cell specific protein or a portion thereof encoded by the nucleic acid of claim 1.
- 20 18. The isolated hematopoietic stem cell of claim 16, further comprising the following characteristic:
- capable of modulating hematopoietic stem cell activity.
19. The isolated hematopoietic stem cell of claim 18, wherein the activity is selected from the group consisting of hematopoietic stem cell differentiation and hematopoietic stem cell replication.
- 25 20. The protein of claim 17, wherein the protein is selected from the group consisting of a growth factor, a transcription factor, a splicing factor, a capping factor, a transport protein, a translation factor, and a replication factor.
- 30 21. The protein of claim 17, the protein having substantially the same amino acid sequence as one selected from the group consisting of SEQ.ID.No.: 42, SEQ.ID.No.:

44, SEQ.ID.No.: 46, SEQ.ID.No.: 48, SEQ.ID.No.: 50, SEQ.ID.No.: 52, SEQ.ID.No.: 54, SEQ.ID.No.: 56, SEQ.ID.No.: 58, SEQ.ID.No.: 60, SEQ.ID.No.: 62, SEQ.ID.No.: 64, SEQ.ID.No.: 66, SEQ.ID.No.: 68, SEQ.ID.No.: 70, SEQ.ID.No.: 71, and SEQ.ID.No.: 73.

5

22. A nucleic acid probe capable of specifically hybridizing with the nucleic acid of claim 1 under standard conditions.

23. An antibody capable of specifically binding to the protein of claim 17 without substantially cross-reacting with a non-stem cell specific protein or homologs thereof under conditions permissive to antibody binding.

24. A cell capable of producing the antibody of claim 23.

15 25. A method for identifying the presence of a primitive hemopoietic stem cell in a sample comprising:

(a) contacting the sample with the antibody of claim 23 under conditions permissive to the formation of an antibody complex; and

20 (b) detecting the presence of the complex formed in step (a), the presence of a complex formed indicating the presence of a primitive hemopoietic stem cell in the sample.

26. The method of claim 25, wherein the antibody is labeled with a detectable marker.

25 27. The method of claim 26, wherein the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, magnetic bead, dye, fluorescent marker and biotin.

30 28. A method for generating a stem cell/progenitor cell from a primitive hematopoietic cell in a sample comprising contacting the sample with the protein of claim 17.

29. A method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity comprising:
- (a) contacting the hematopoietic stem cell with the sample;
 - (b) determining the hematopoietic stem cell activity;
 - 5 (c) comparing the hematopoietic stem cell activity determined in step (b) with the activity determined in the absence of the compound an increase or decrease in hematopoietic stem cell activity indicating the presence in the sample of a compound that modulates hematopoietic stem cell activity.
- 10 30. The method of claim 29, wherein the activity is selected from the group consisting of gene expression, replication, differentiation, transplantation, and self regeneration.
31. A compound identified by the method of claim 29, previously unknown.
- 15 32. A method for identifying primitive hematopoietic stem cell-specific nucleic acids , comprising:
- (a) creating a primitive hematopoietic stem cell cDNA library and a non-primitive stem cell immune cell cDNA library; and
 - (b) subtracting the two libraries, thereby identifying primitive stem cell specific
20 nucleic acids.
33. The method of claim 32, step (b) comprising:
- (i) contacting the nucleic acids of the stem cell and non-stem cell libraries with each other under conditions permissive to hybridization, thereby forming
25 hybrid complexes;
 - (ii) separating the hybrid complexes formed in step (b) from the nucleic acids which did not form complexes;
 - (iii) isolating the nucleic acids which did not form complexes, thereby identifying hematopoietic stem cell specific nucleic acids.
- 30 34. The method of claim 33, step (ii) further comprising amplification of the nucleic acids.

35. The method of claim 33, step (iii) further comprising amplication of the nucleic acids which did not form complexes.
36. The method of claim 33, further comprising displaying the amplified DNA on a chromatography gel.
37. The method of claim 32, step (b) comprising differential display of the two libraries, thereby identifying primitive stem cell specific nucleic acids.
38. The method of claim 32, step (b) comprising representation difference analysis of the two libraries, thereby identifying primitive stem cell specific nucleic acids.
39. The method of claim 32 further comprising cloning the stem cell specific nucleic acid.
40. The method of claim 32, wherein the stem cell is selected from the group consisting of AF024, 2012, and 2018.
41. A nucleic acid identified by the method of claim 32
42. A composition comprising the compound of claim 31 and a carrier.
43. A pharmaceutical composition comprising the compound of claim 31 and a pharmaceutically acceptable carrier.
44. A primitive hematopoietic stem cell specifically expressing one selected from the group consisting of: SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25, SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39,

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SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a functional portion thereof.

5

45. A primitive hematopoietic stem cell specifically expressing a nucleic acid identified
by the method of claim 32.

10

46. A method for treating a condition in a subject comprising administering to the subject
a therapeutically effective amount of the composition of claim 43.

47. The method of claim 46 wherein the condition is an immune system condition.

48. The method of claim 46 wherein the condition is leukemia.

15

49. A method of introducing an exogenous nucleic acid into a hematopoietic stem cell
comprising contacting the stem cell with the composition of claim 42.

20

50. A method of ex vivo expansion of hematopoietic stem cells comprising contacting the
cell with the composition of claim 42.

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FIGURE 1

Figure 1. Subtracted Libraries

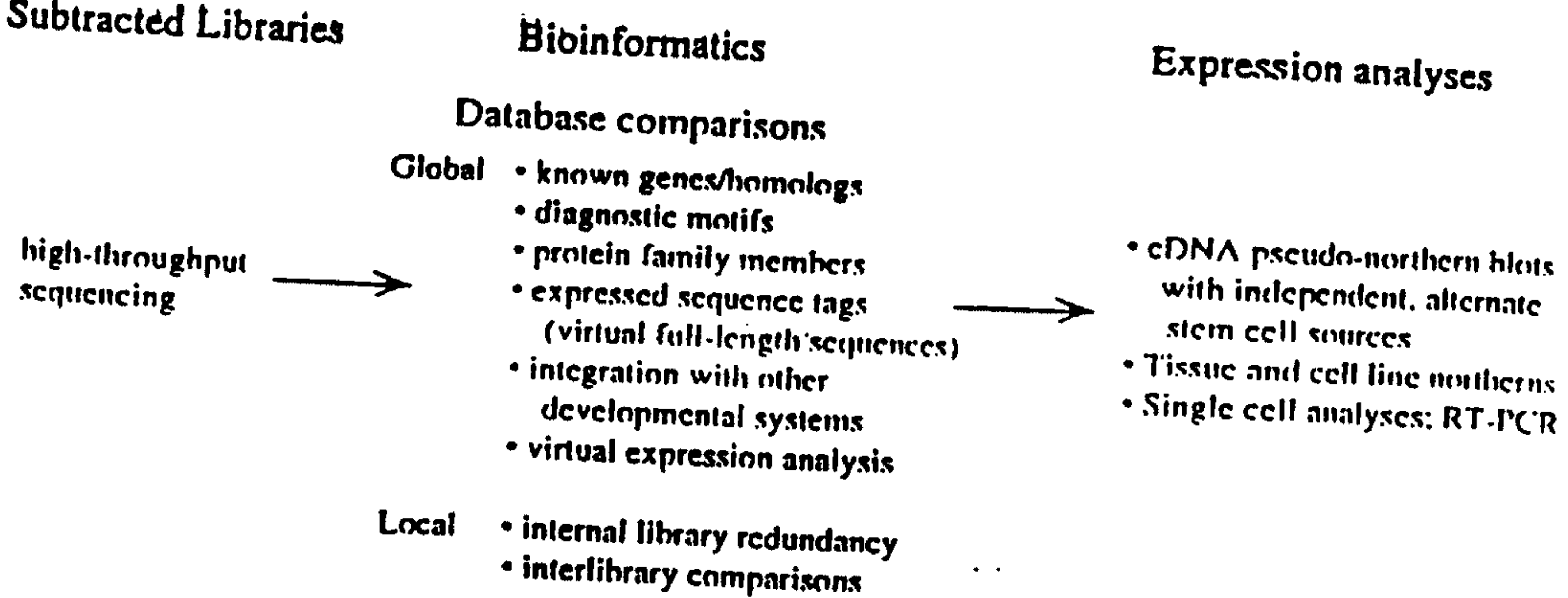


FIGURE 2

Figure 2.

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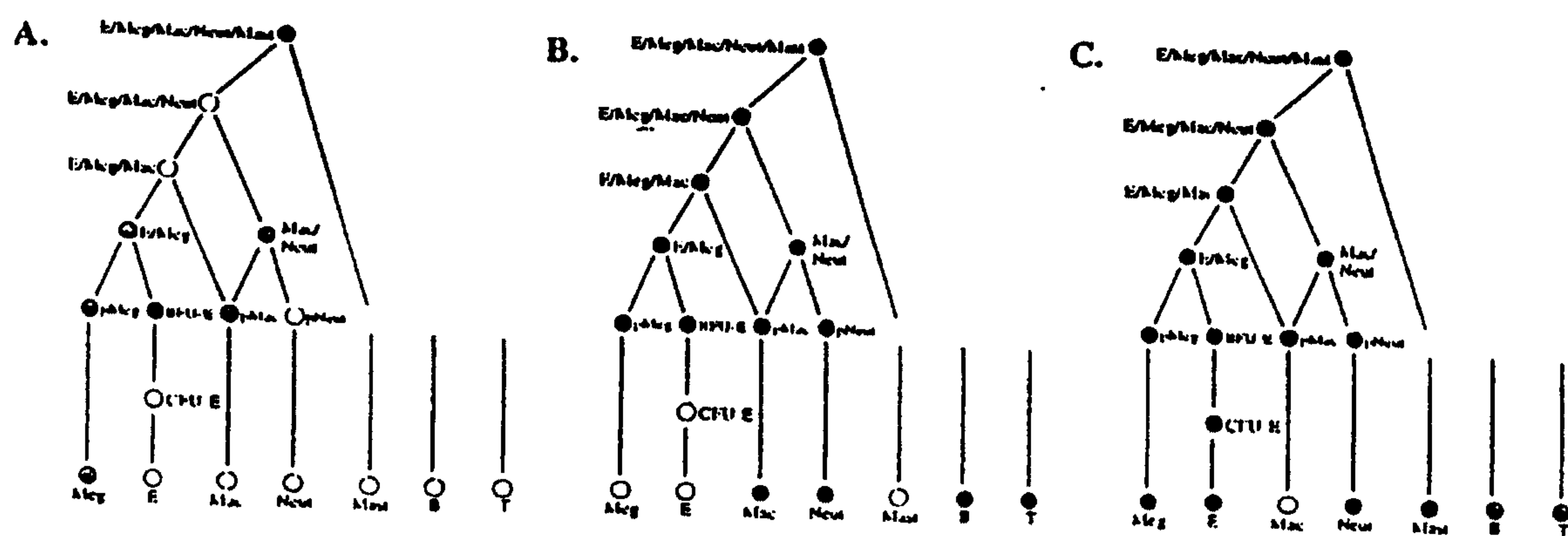


FIGURE 3

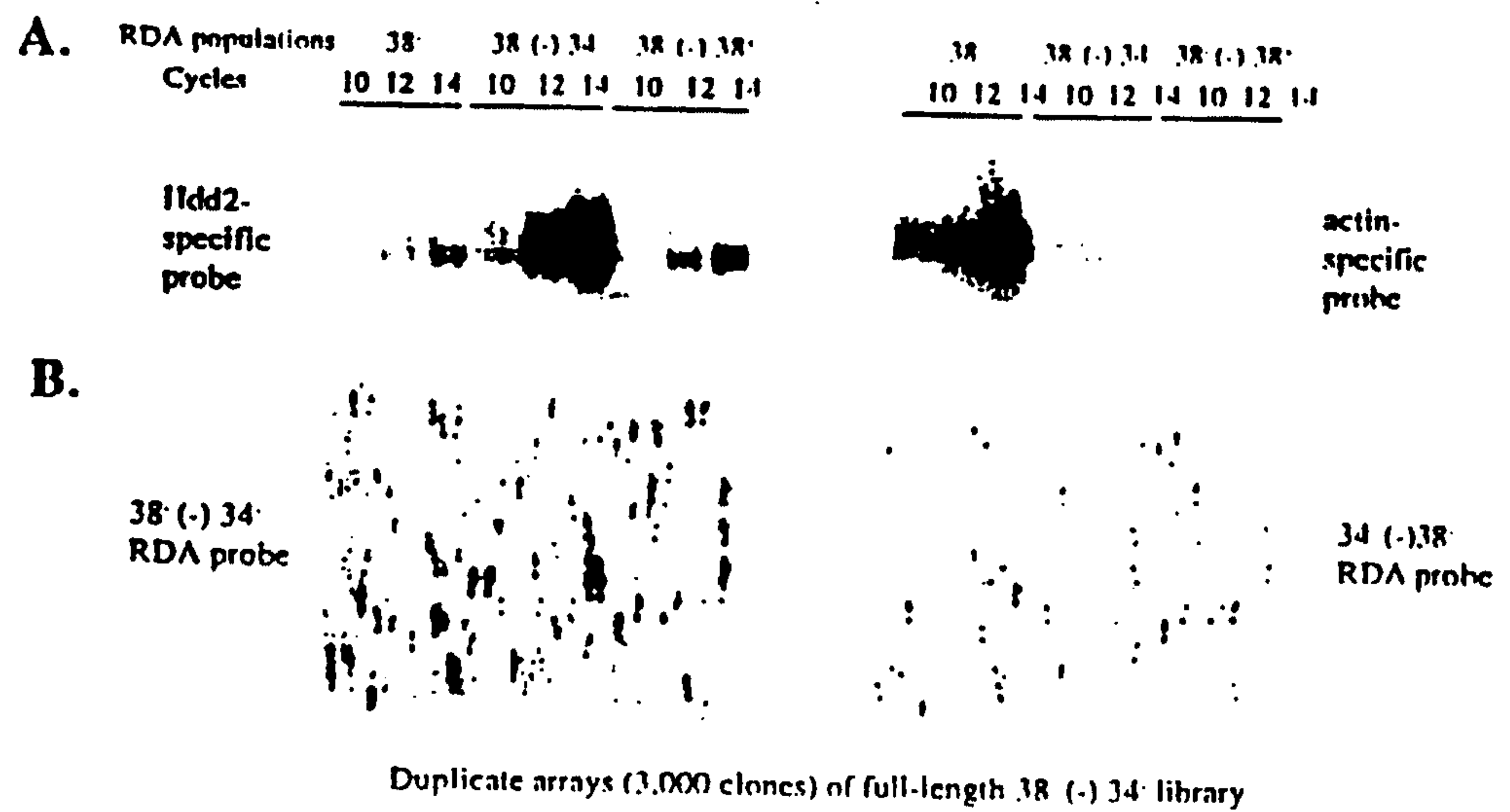


FIGURE 4

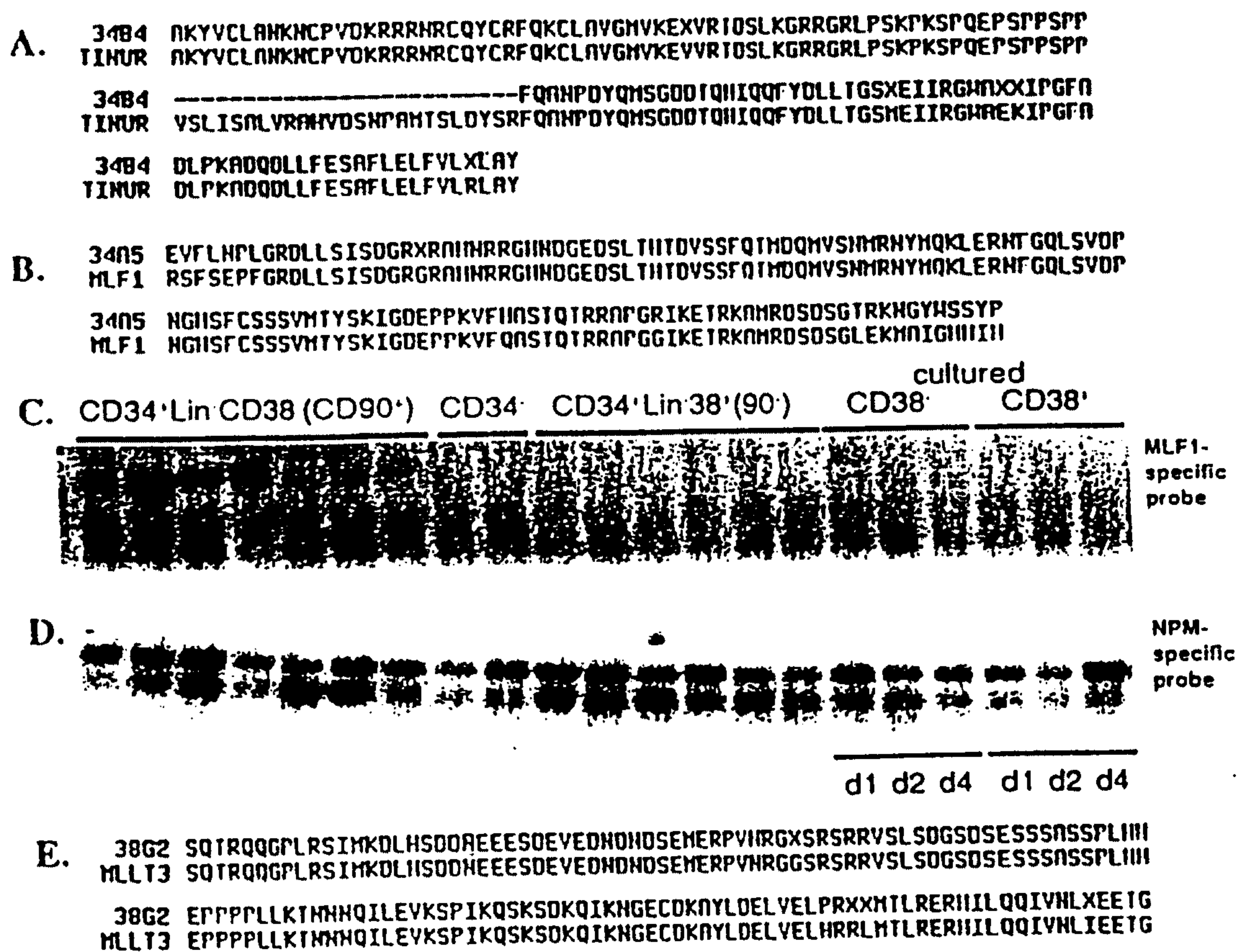
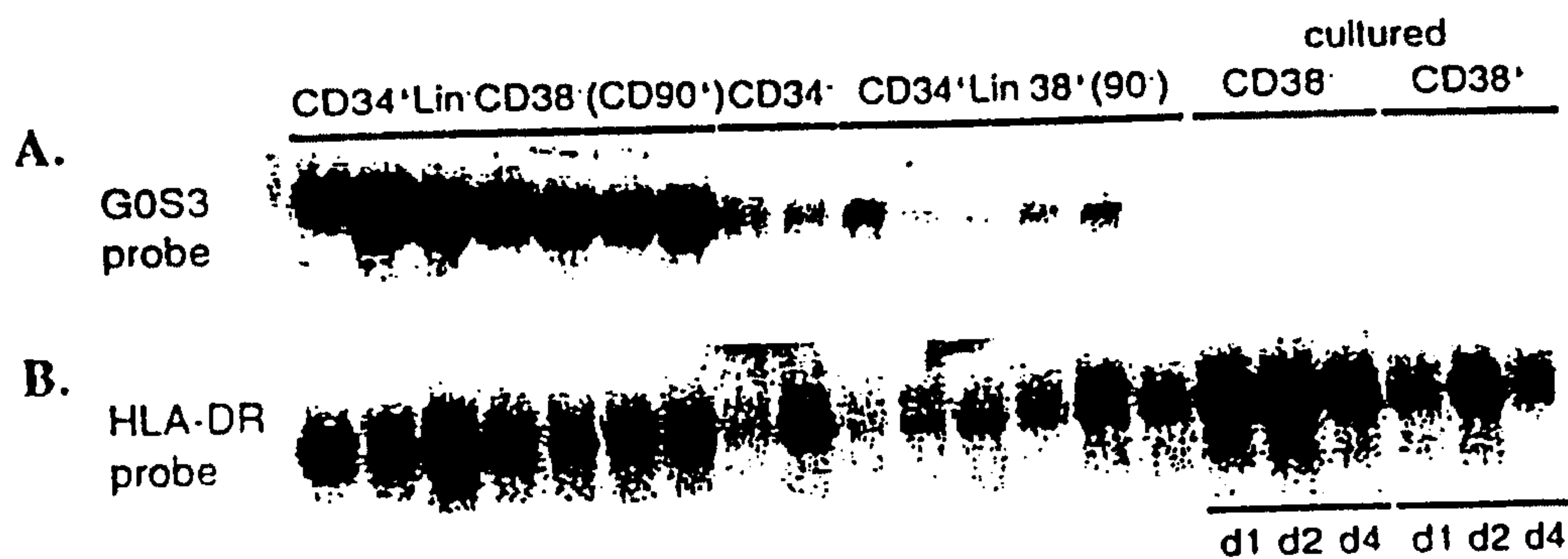


FIGURE 5

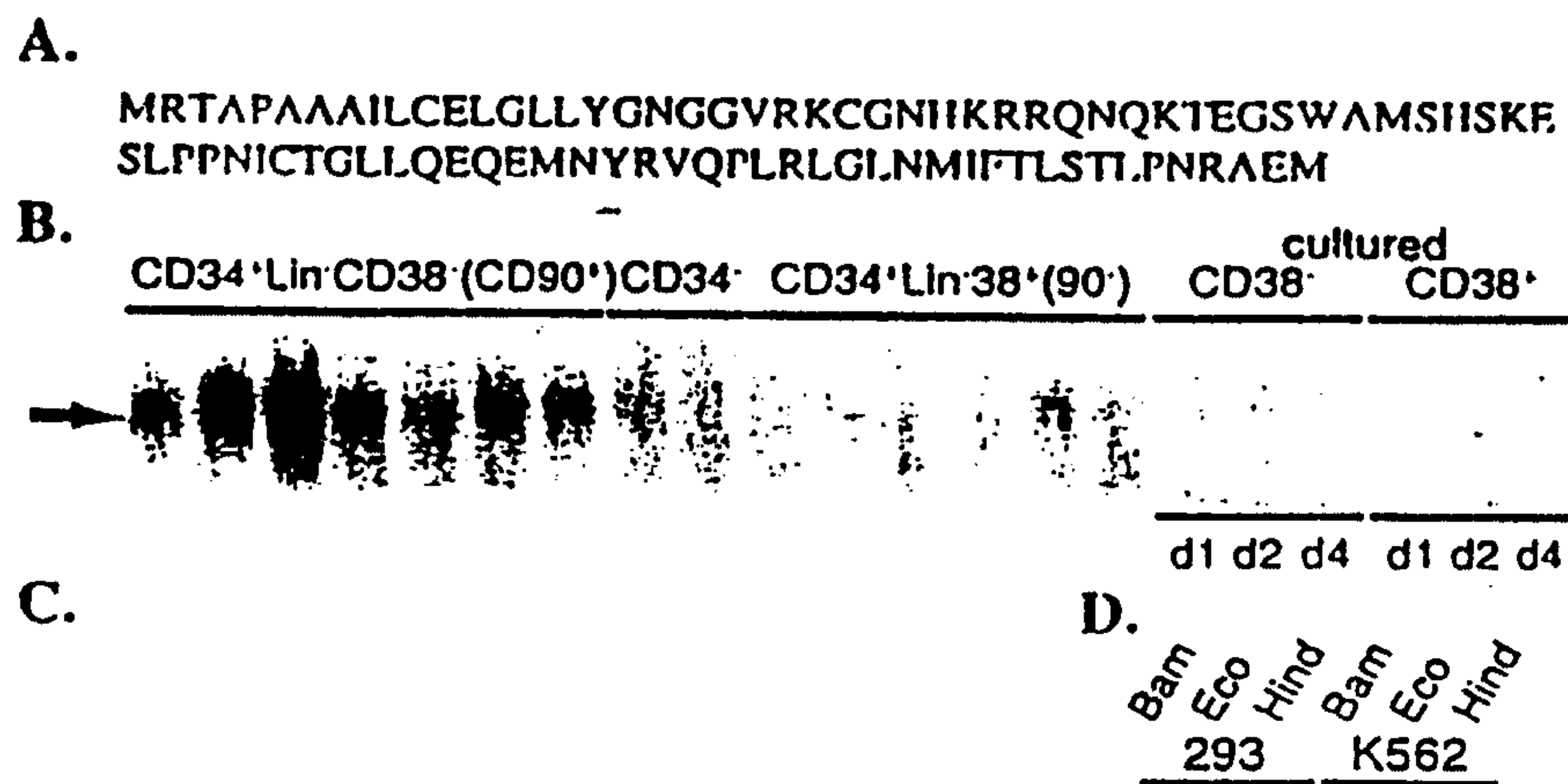
Figure 5.



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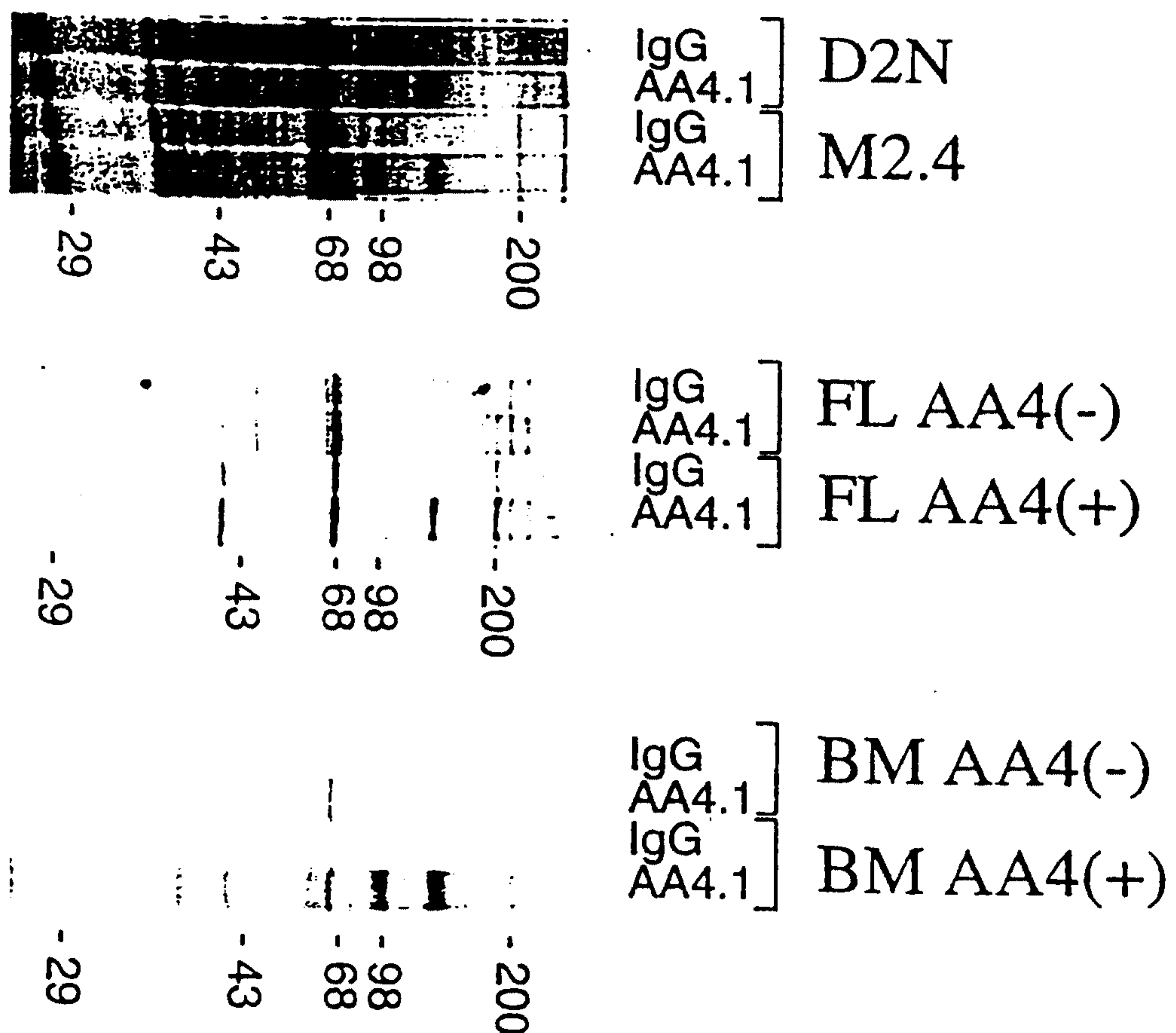
FIGURE 6

Figure 6.



kidney →

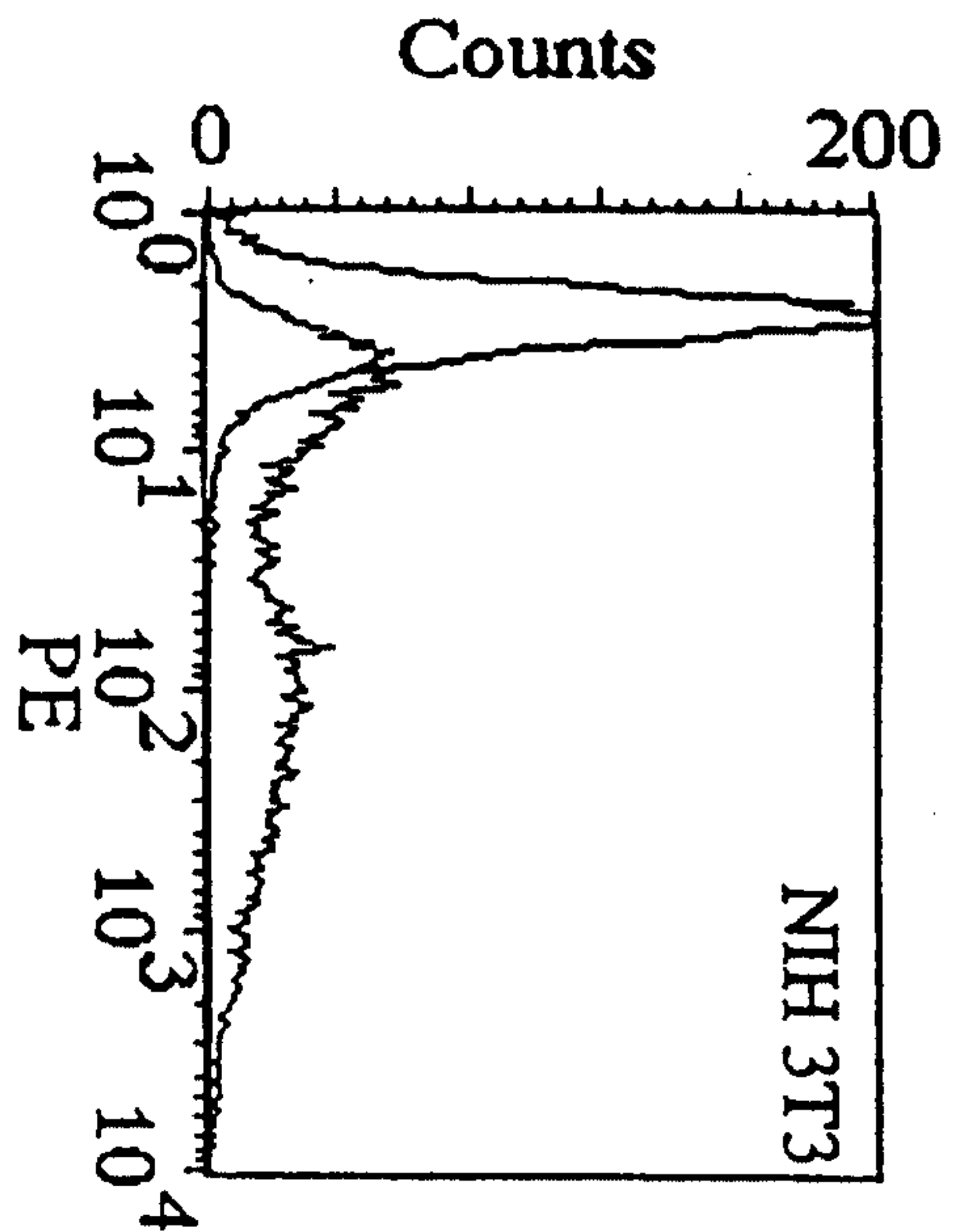
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FIGURE 7

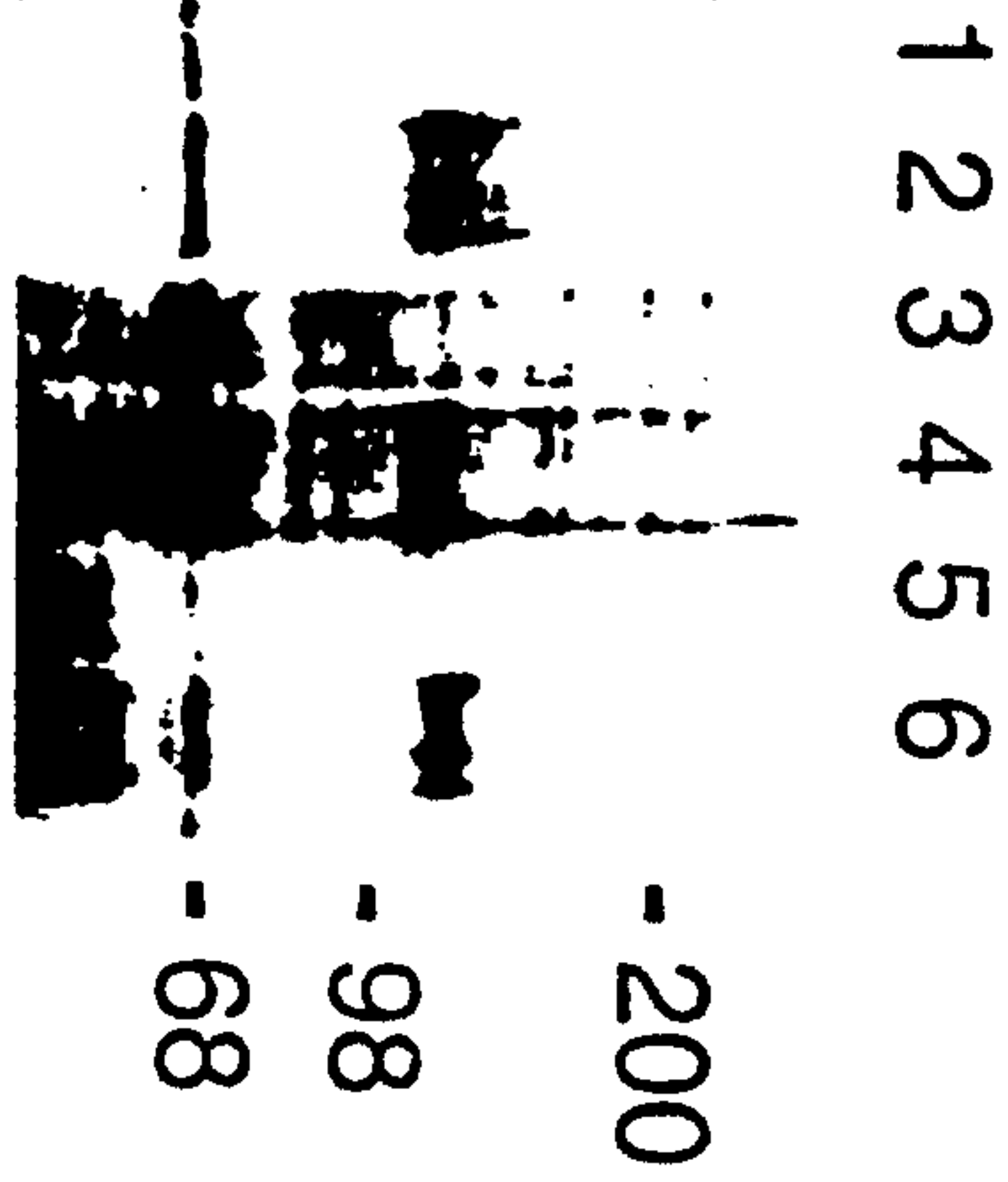
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FIGURE 8

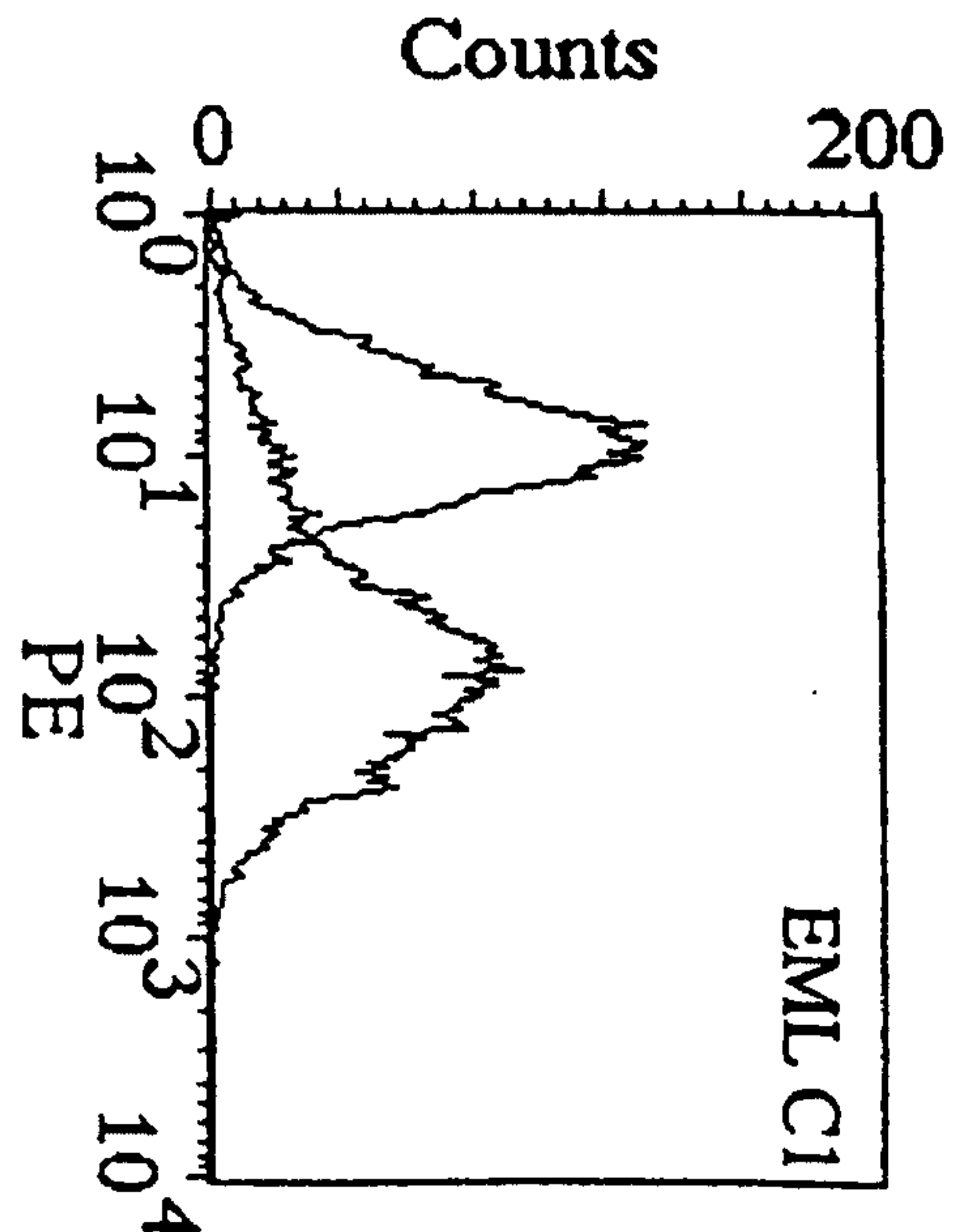
A



B



C



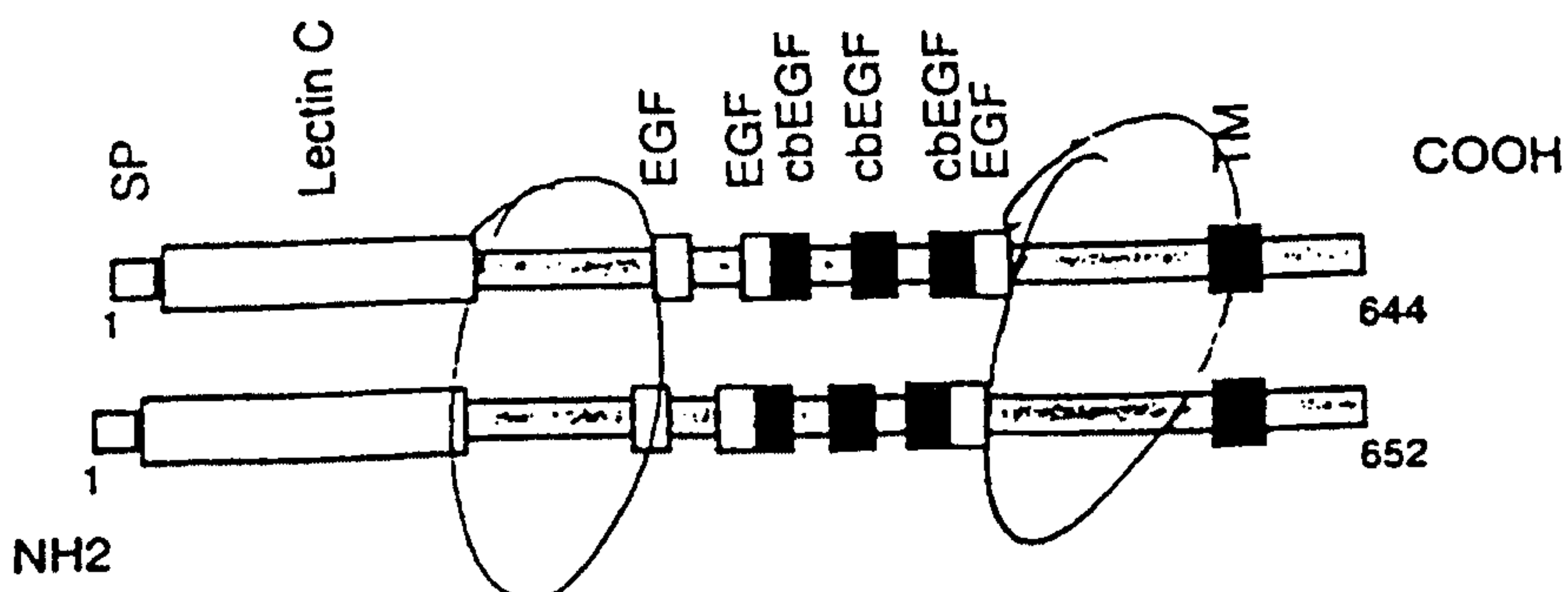
1 2 3 4 5 6 7 8



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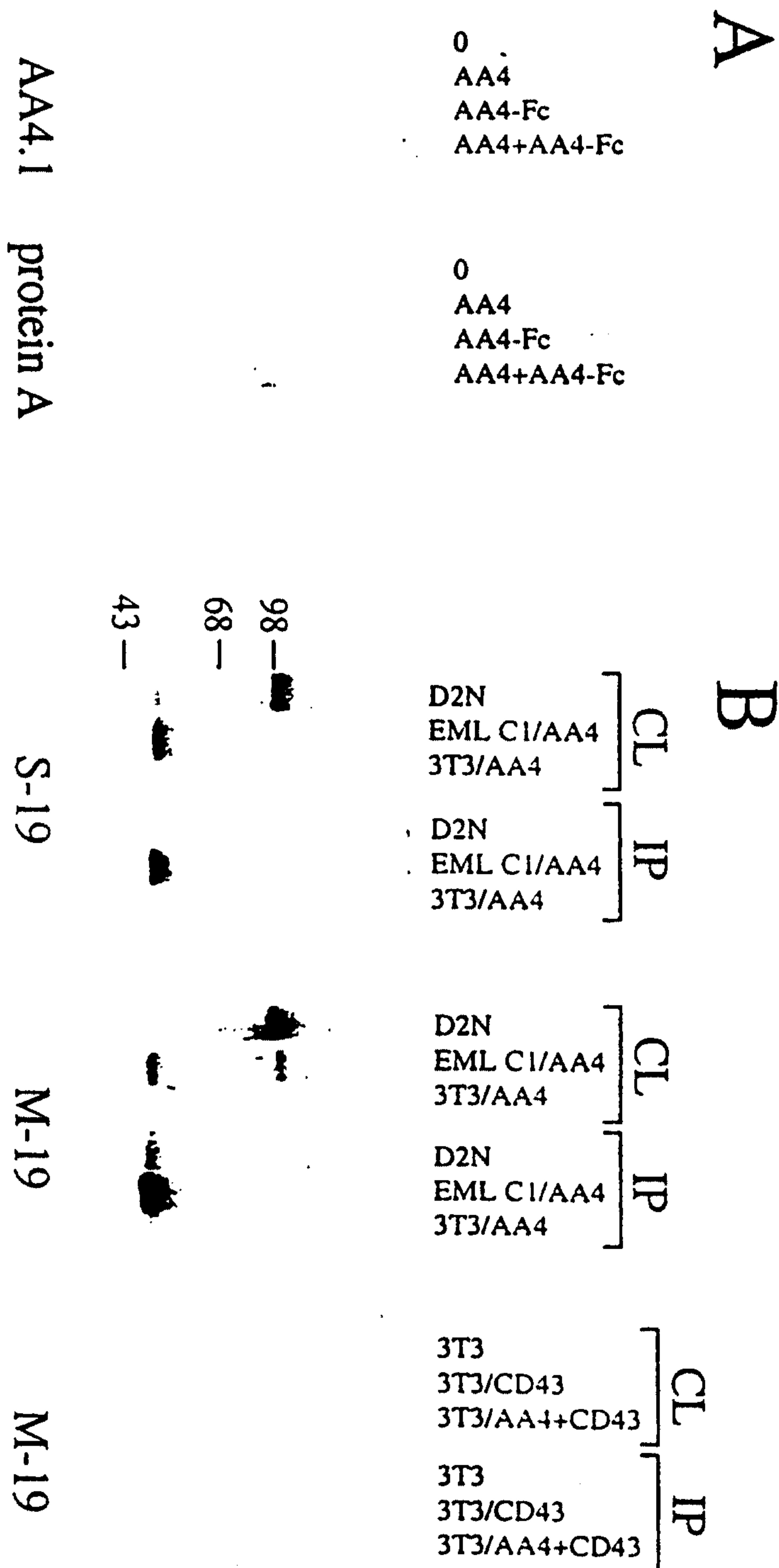
T U U T V

[illegible]



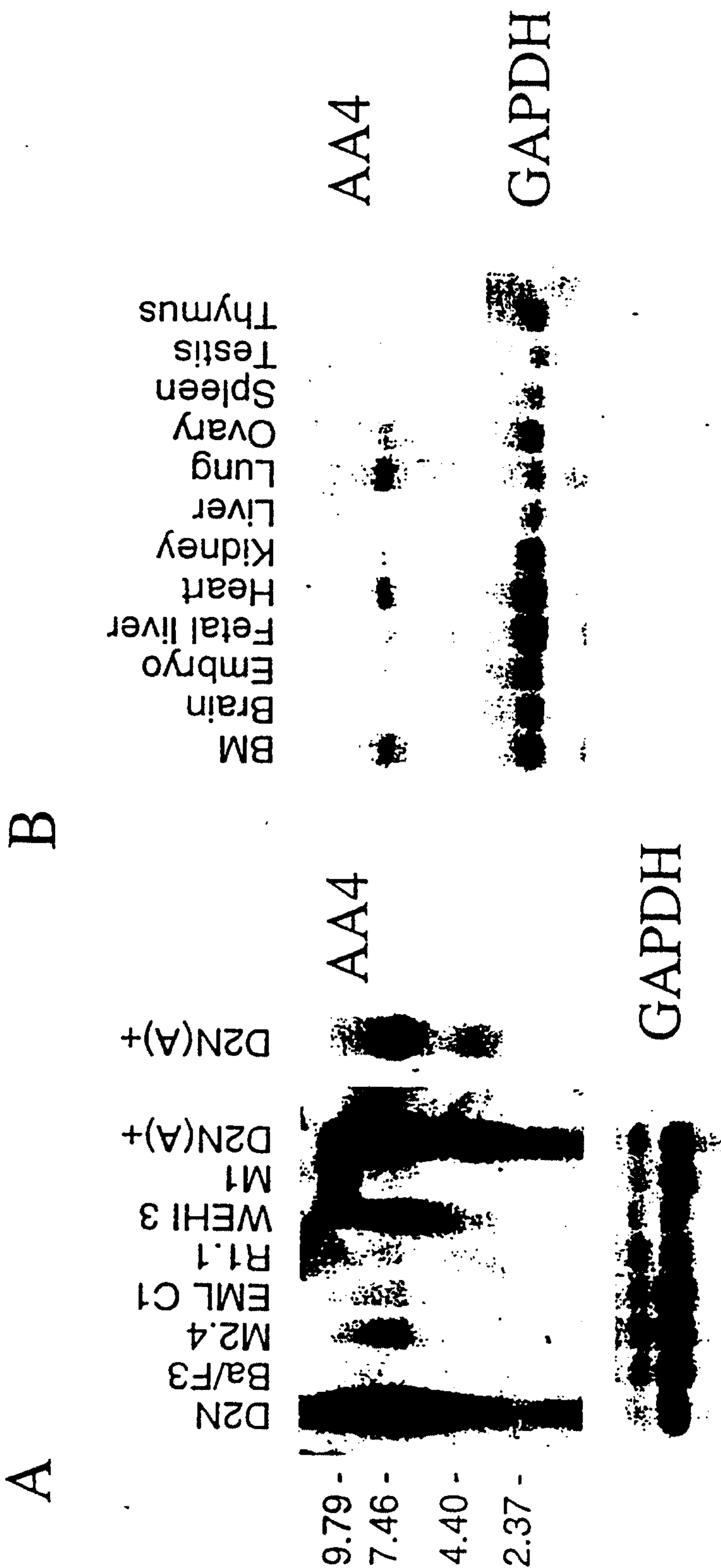
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FIGURE 11



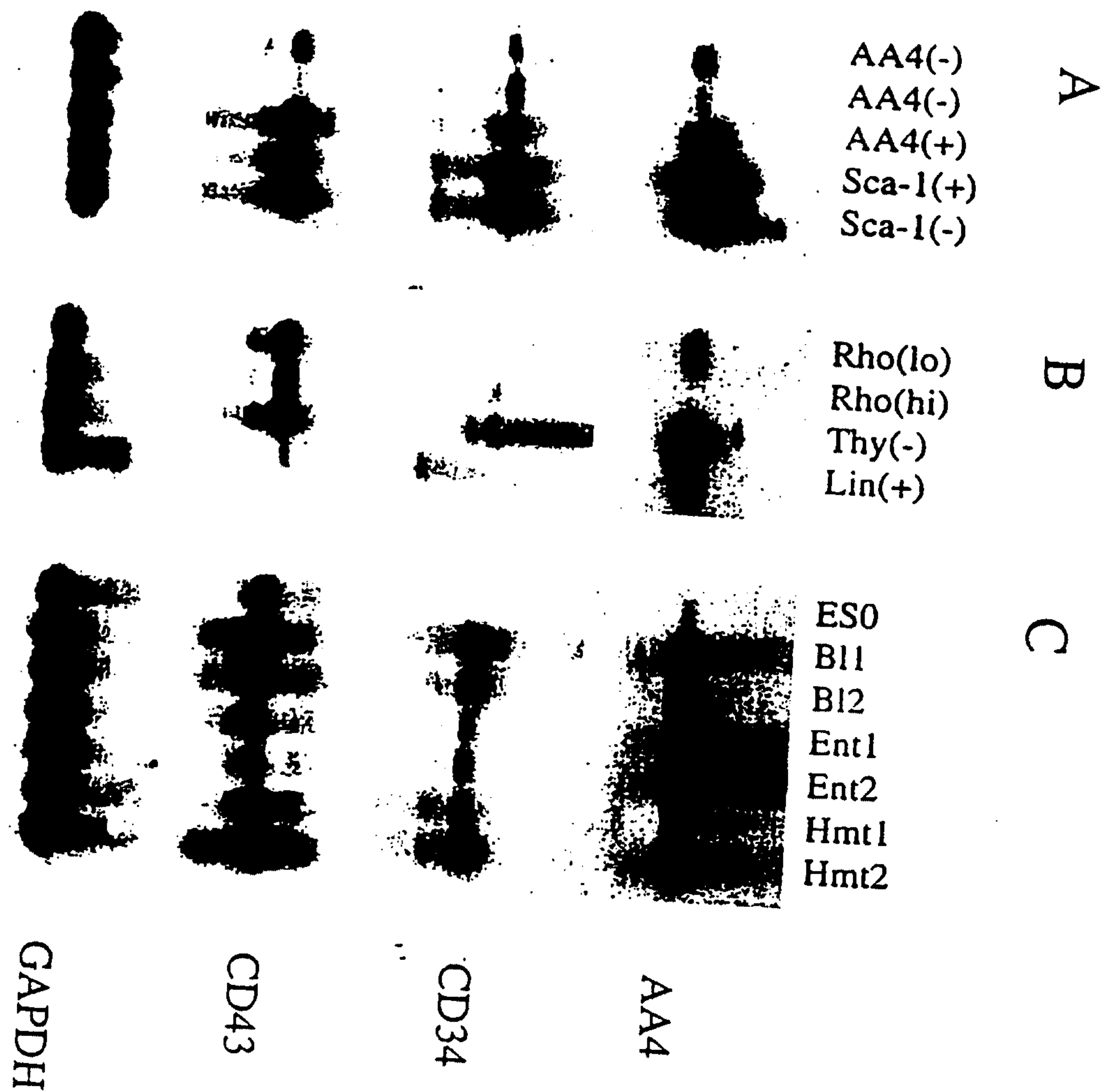
12/99

FIGURE 12



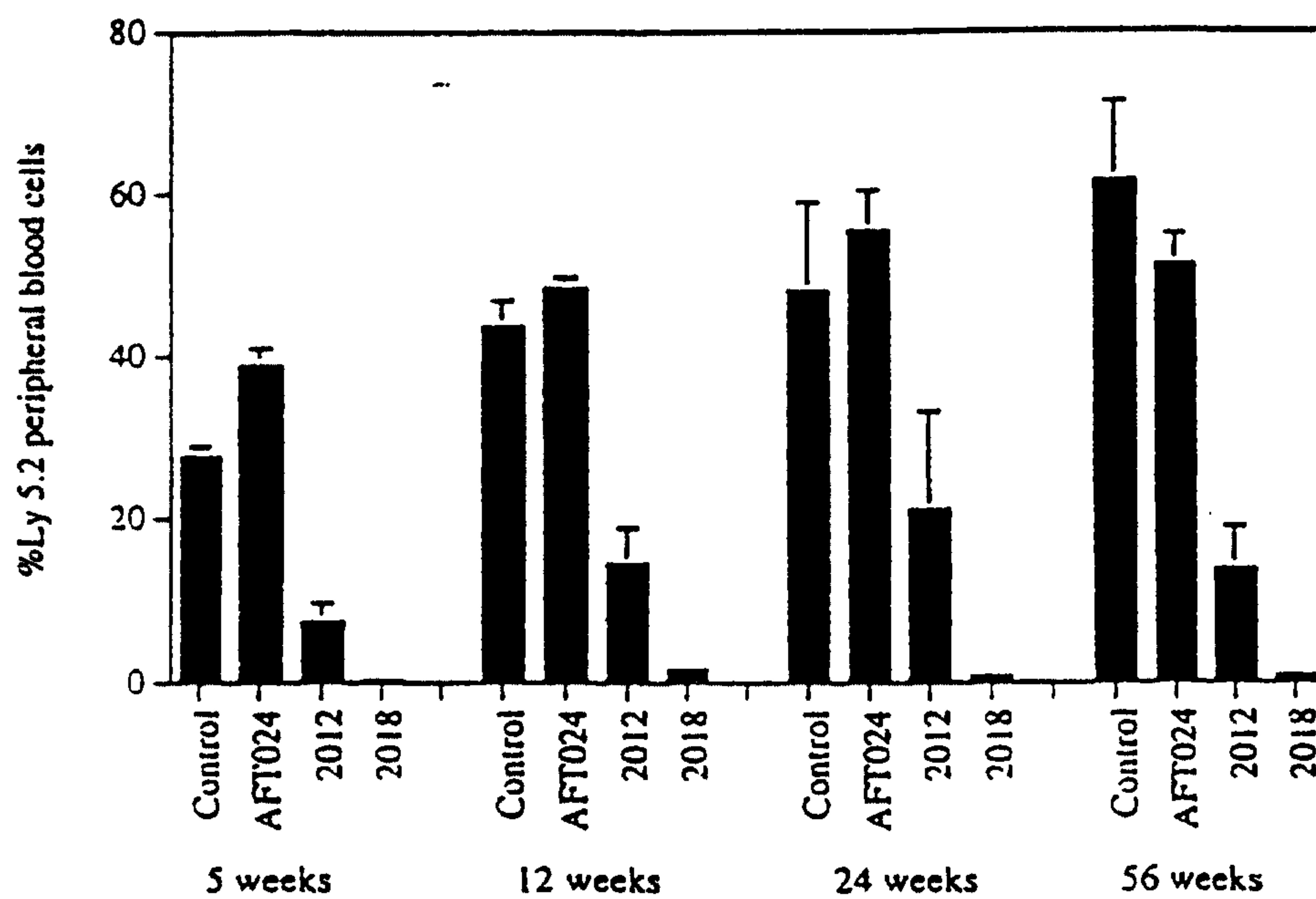
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FIGURE 13



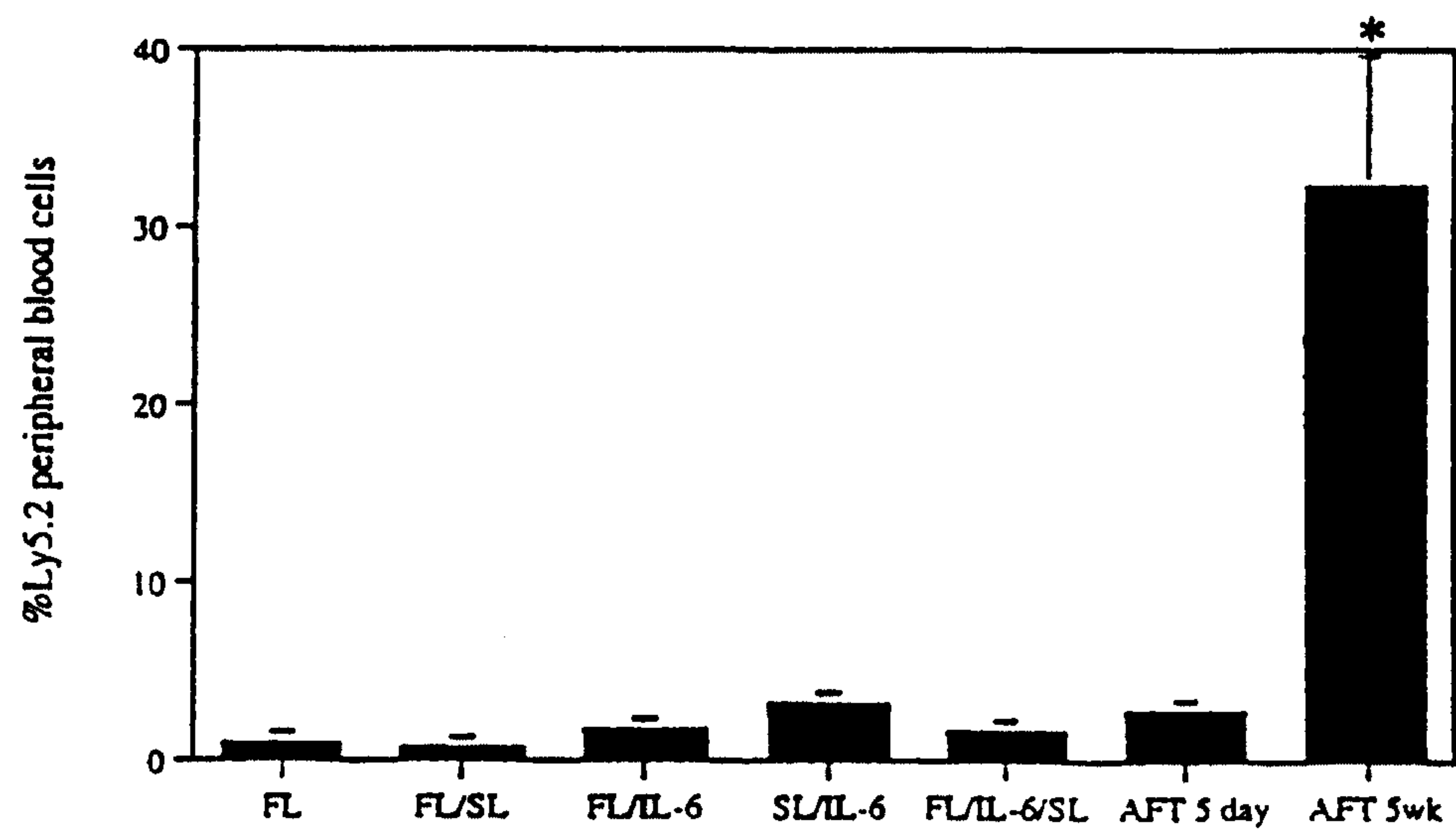
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FIGURE 14



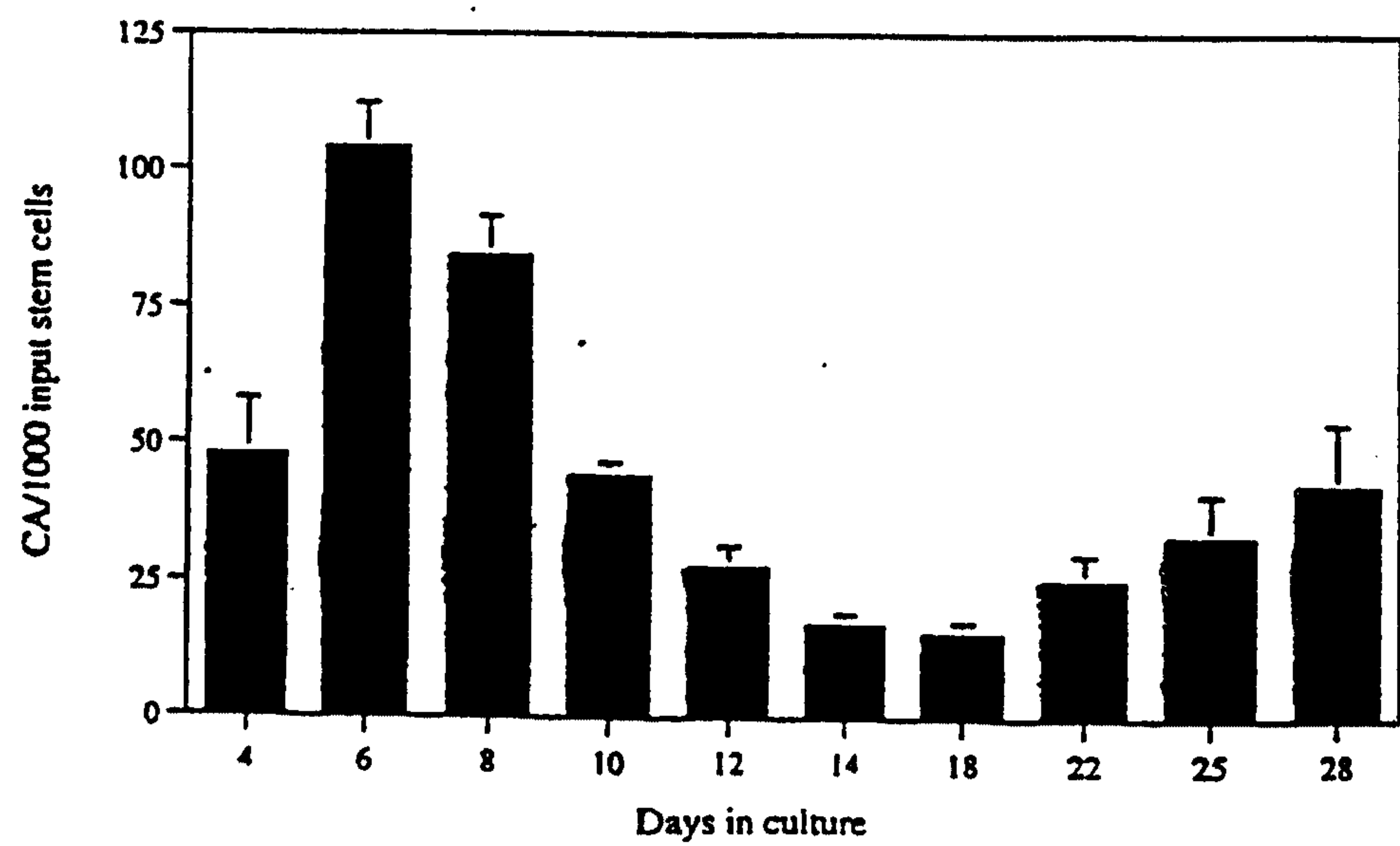
15/99

FIGURE 15



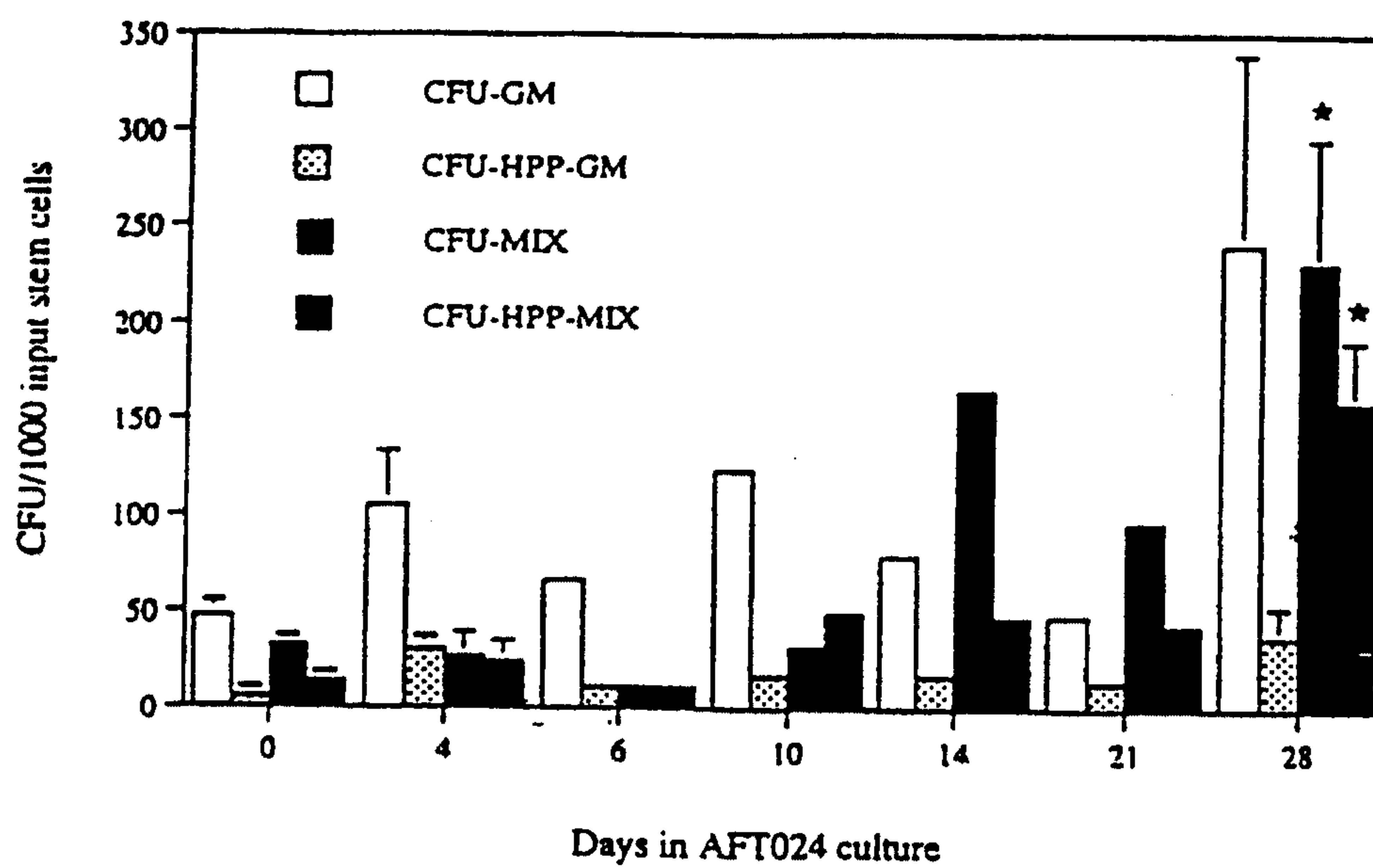
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FIGURE 16



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FIGURE 17

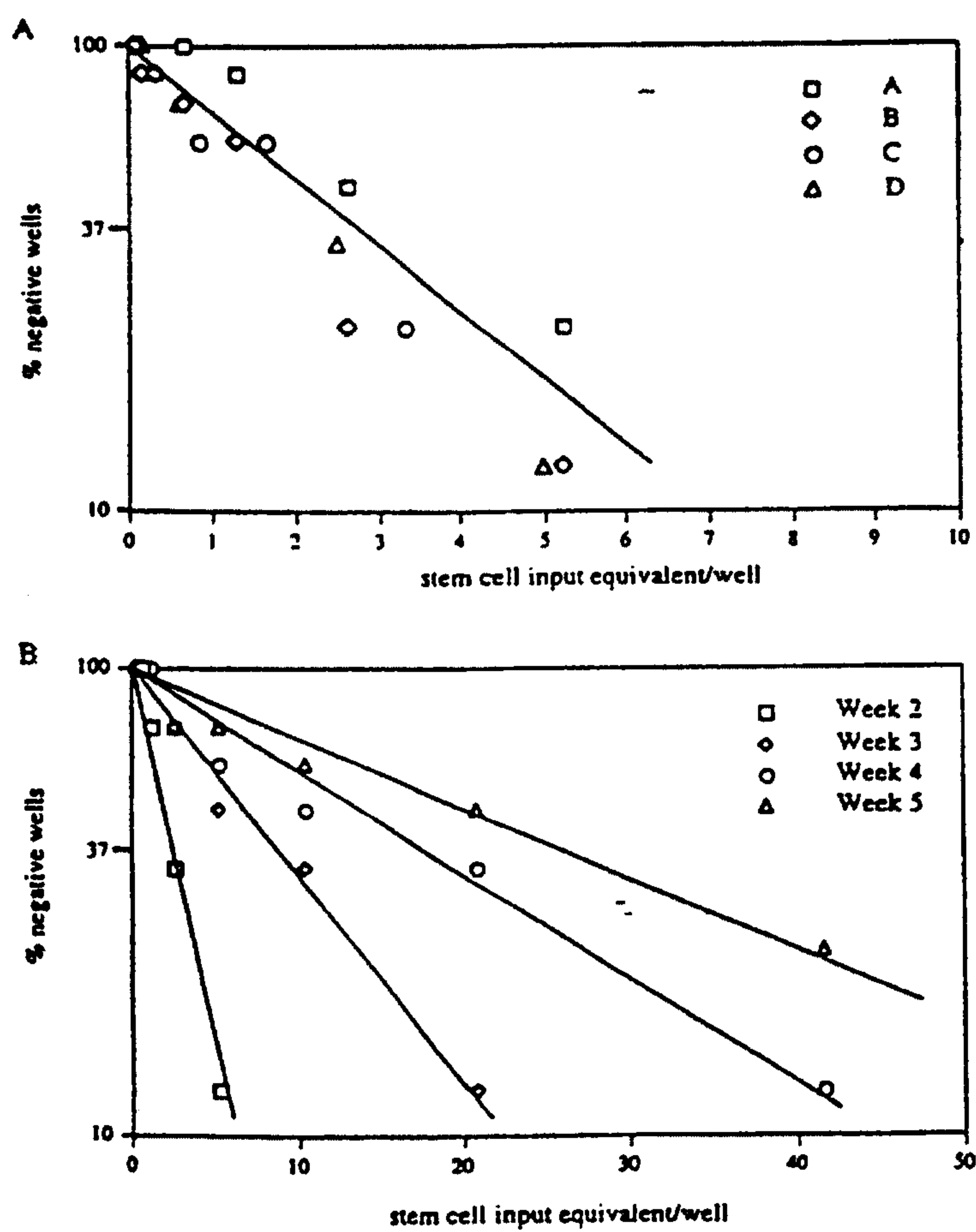


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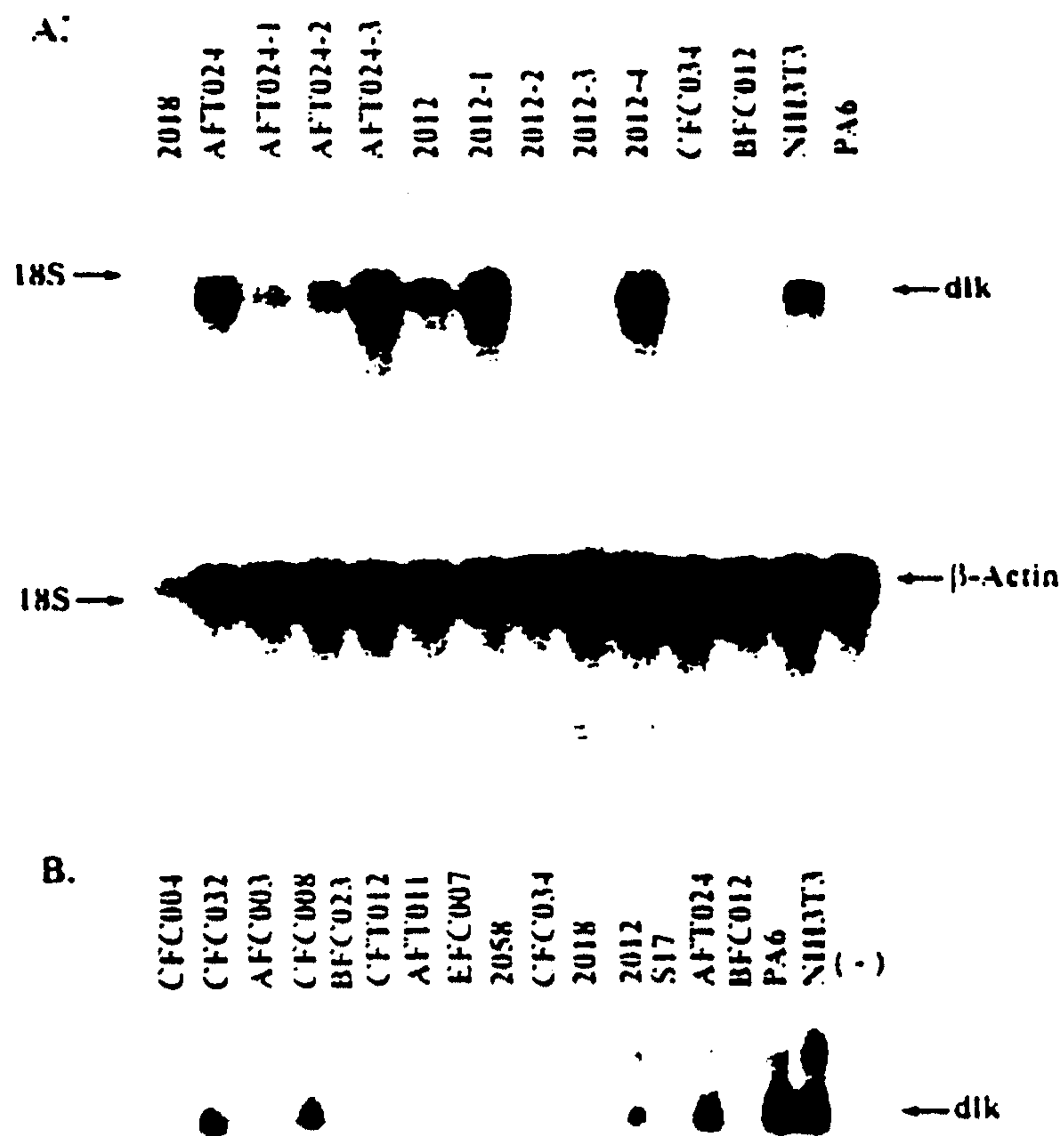
PCT/US99/19052

FIGURE 18



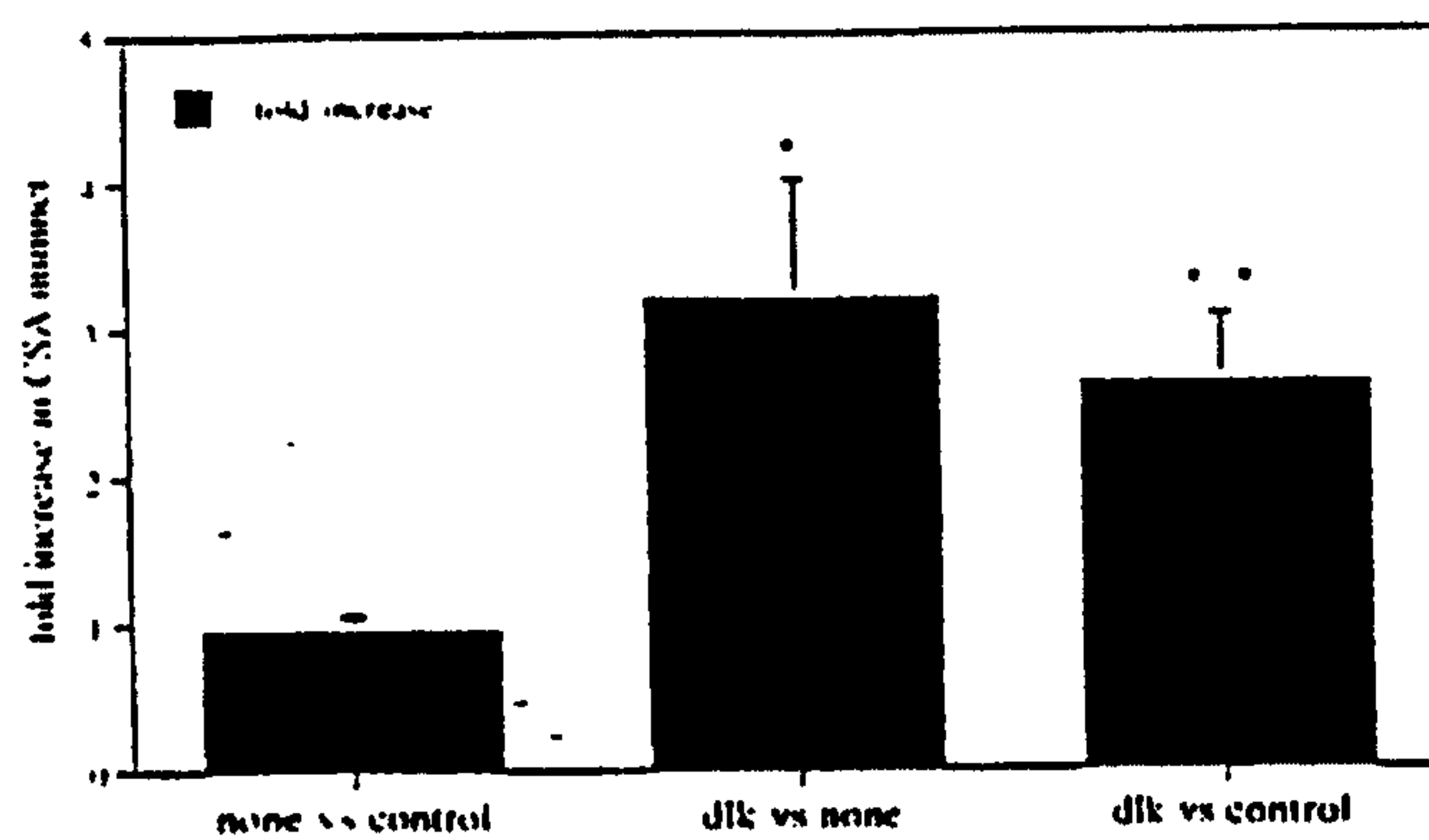
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FIGURE 19

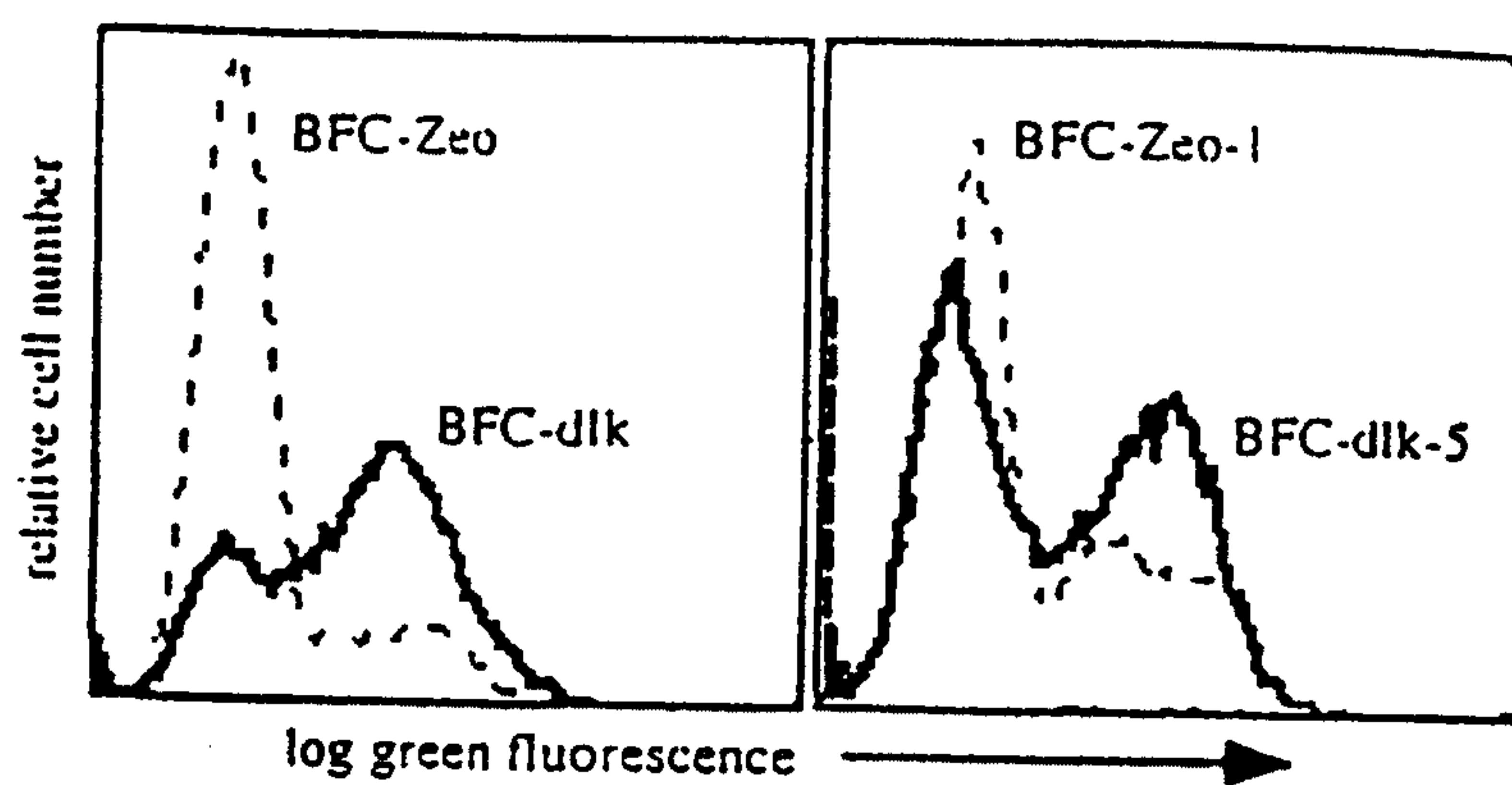


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FIGURE 20

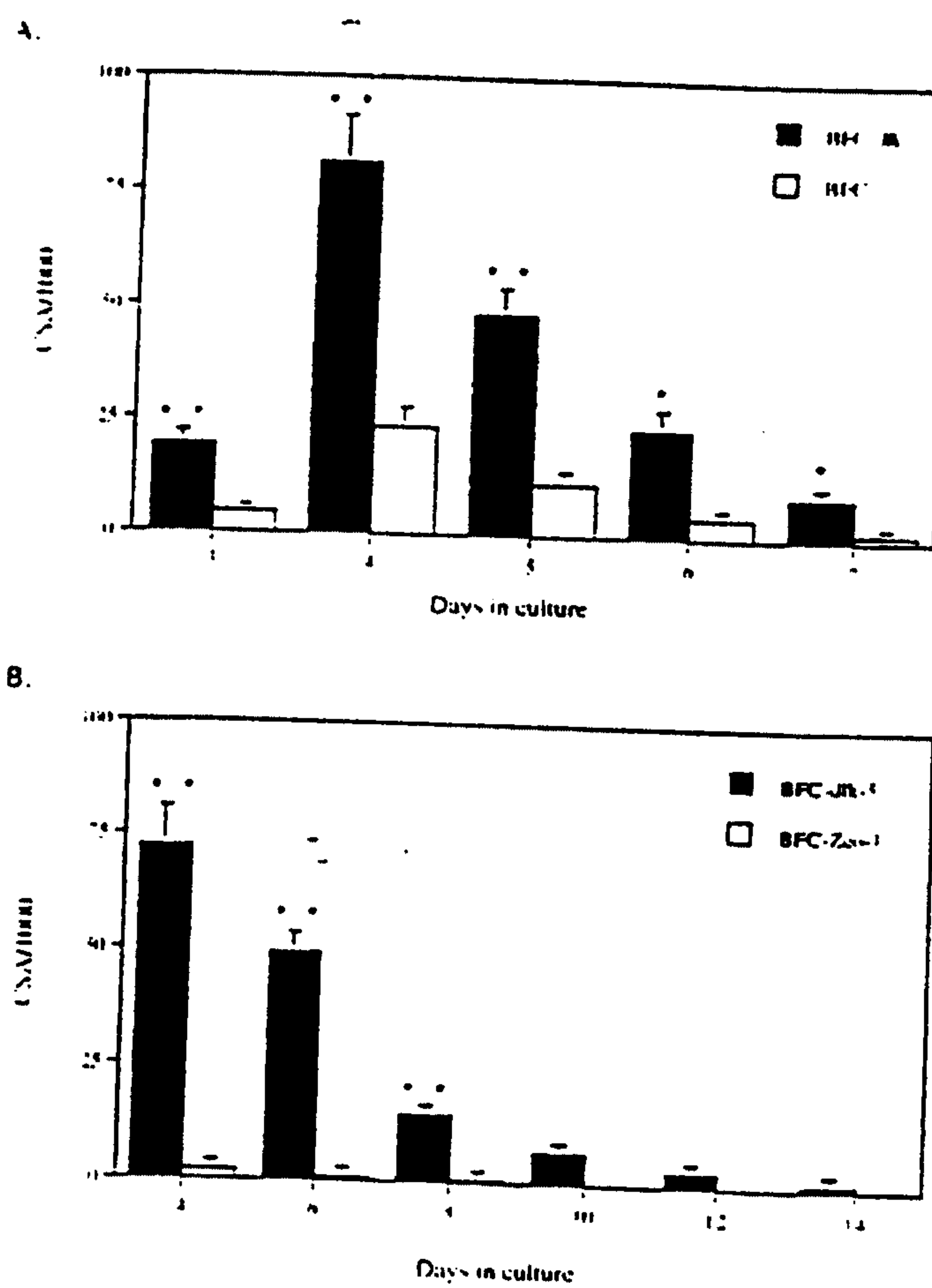


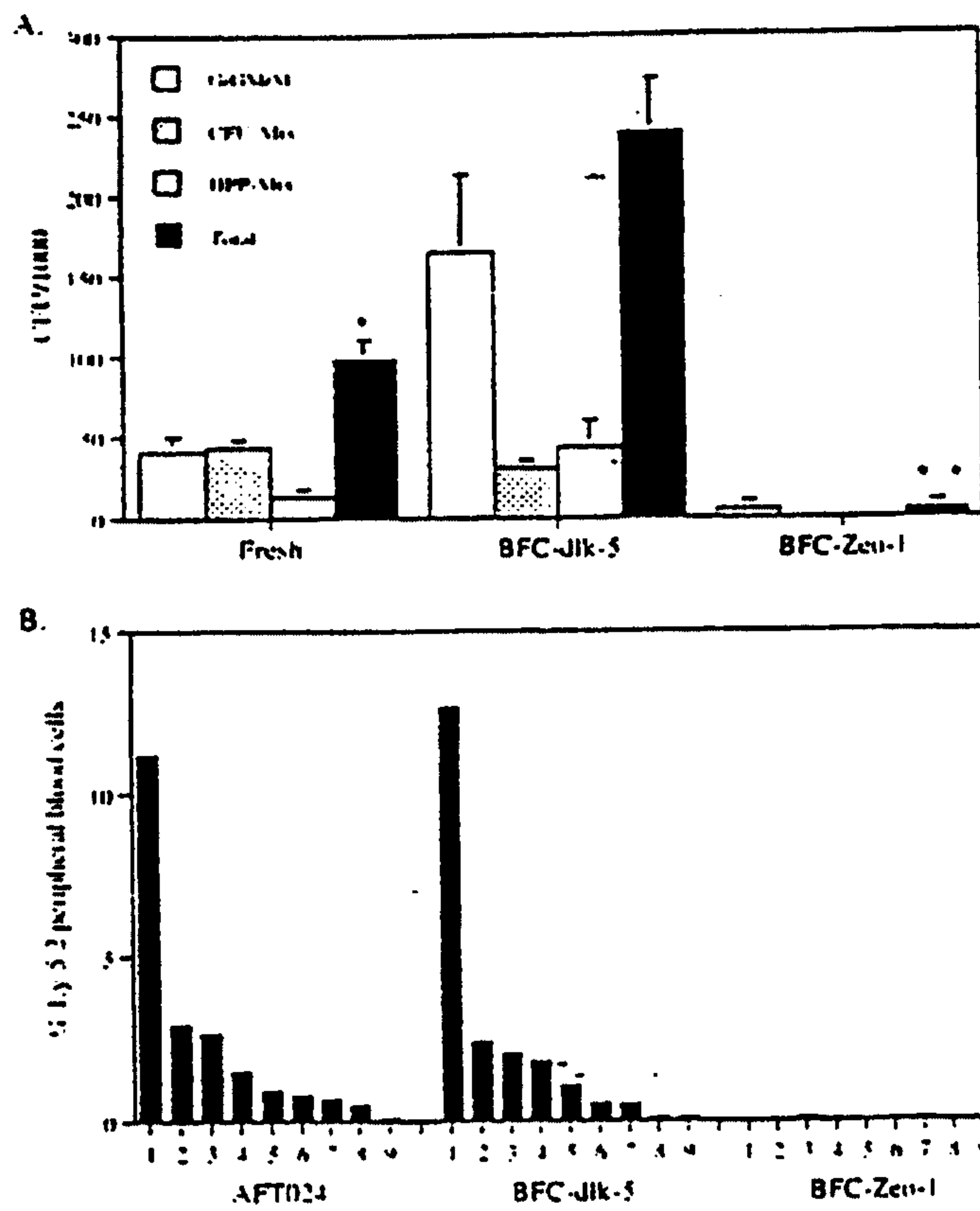
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FIGURE 21

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FIGURE 22





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-- LL4-39 (0134)

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Page 1

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1  CCCAGCCTC: CCGAGAGCCG CAGCCATCTC CATGCCAAGG TTGAACAAA
51  TCCAGGAA: GTTCTTTTCT CCTAGCCCA AGGATTGGA AGAACACAGC
101 CATTCTTT: TAGGCTACAA AAGCAGAAA AAAAGGCCAA AGACTTTTGG
151 AATGACCT: AAACATACC TGAGATCGAT GATCCACAT CTGGAATCTG
201 GATGAAAT: GGGCAATTC AAGACATAC TTTCTCTGA AGAAGTTATG
251 CAGTCTCT: AGTCTCTGA AAAATCCTG CCAACCAGAC AGGTCTGAA
301 GTCTTTGA: GATCTGAAG TCTGATTCAG TGAGGAAT ATTGAATTCT
351 GGTTCCTT: TGAGACTAT KAKAAACAN AACTGATCT TTGCATAC
```

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FIGURE 24

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23.41 (D194)

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1. CCGCTTCGAC AAGACGACAG AAGCGGACTC ACTCGTTTTC AGAAGACCAT  
5. GANAGCGGCA GCGTCTCCA TCCCAAGGTT GACCAAAATG CCAAGGATGT  
101 TCTTTCTGTC TATCCGAAAG GATTCGAAAN AACACAGCCA TTCTCTTC
```

SEQ ID. No.: 2

FIGURE 24

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Bl-66 (related to G03B)

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1  TACGGCTGCT AGAAGACAGC AAGAGGCGAA NCAGCTGCGG TGCGCGCGCG
51  AGTCTGACAA TGCAAGTGC CATGTTCTCT GCTGTCGCGC AGGACTGCGT
101 ACCCATGGA: AAGAGTGCAN GCAACGGCCC CAAAGTCHBN GAGAAAGCGG
151 AGAAATGAA TCGGACACTC TTTAAGATTT GGAAGAGCCC TTTGAGCTAC
201 TCTGACAGA GAGCTCTCTG TCGCGGGAAG CCGTGAAGTG GCAATAAAC
251 KACAGCATA CTTTATATTA NCCTTCTCTT KAGAGAGCGC ANCTCTAGC
301 ATAACTTT: NATGAGCTGC TGCGCGTAT ATATGCGCTG GCTGATTA
351 NGCGTTTT: TAAGTCCCA GTTCTGTGA GAAACATTE AATTCTGCT
```

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FIGURE 24

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LL2-12 (brinac)

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Page 1

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1  CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG
51  CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG
101 CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG
151 CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG
201 CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG
251 CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG
301 CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG
351 CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG CCTCTGCTG
```

SEQ ID. No.: 4

FIGURE 24

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665-96 (lin) obl

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Page: 1

1 CCCAGCCTC CGATCTCTC CAGGCCACC AAGCAGCTT GAAGAGCCAT
51 GTTCGAAAC: OCCGAGCCG CCCAGGCCAC CCGCTCTCAT GAAGCCAAAG
101 GCAGCAGTC: CAGCAGCAGT GTACAGCCGT CTAAGTCCTT TACCTTCGG
151 CCTCAGCTGA AAGAGACCTG TGCAGCCTGC CAGAGACTG TGTACCCGAT
201 GGAGCCGCT: CTGCGAGACA AGCTCATTTT CCACAACTCT TTTTCTGTT
251 CCAGAGACT: CCACACCAA CTCAGCCTGG GCACTTATGC TCAATGCAC
301 GGTGAATTT: ACTCAGAGC TCACTTTCAG CAGCTTTTA AGATTAAAG
351 CAACTACGAT GAAGTTTGG TGTAAACAG CACAAGACT CTGCGCCAC

SEQ ID. No.: 5

FIGURE 24

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. 61.5.-96 (line) 35

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1  ATTGOTMCO: CTGCACGAC CCGTCCGAA TTCCCGGTC GACCACCGT
51  CCGCCUCCO: GTCCGATCTC (TCCAGGGG CACCAAGCAC CTCTGAAGAA
101 CATCTTCAA OCTOCCGAA (TCCCGCAGC CACCCCTCT CTGGAAGCCC
151 AATGCAKCA TOCCAOCAGC ACCTTNCAGC GGTCTAAGTC CTTTAACTTG
201 CCGCTCAGC TTAAGTAAAC CTCTCAGCC TCCCTGAAA CTCTCTNCCC
251 NATGAMJCG CTCTGCGCAG ACAACTCTT TTCCAGAAC TCTGTCTCT
301 GTTCGAACA CTCCACACC AACTCAGCC TCGCAGTTA TCTCTCTG
351 CACCTTAAAT TTACTCAG ACCTCACTTT CAGCAGCTGT TCTGAATTA
401 AGGCTTAC NATGAAAGT TTCTCTCTA ACAGCAGAA GAGCTCTGG
451 CCGCTGAGG ACTTACTCA GCGCTNANAN AGCTGANA CCGTTAACA
501 CCGATTCTCT CCGCTCAGT TCTCTCCCTC TCGCAGTTG GAAAGAAAA
551 TAACTCTCTG CCGCTCGGTT (TGAAGAGAA AAGGTCTCT CCGCTCGGTT
601 TTCCCGCTA AGGCTCTCTC CCGTAACTCC TTCTTTTNC CCGGGAAGT
651 TTCTGTTTCT GAGCCCAAT TGAATTTCC CCGTCTCTN TTCTTTTCT
701 CCGCTCTCT CCGCTCTCT (TCTCTCTA AGGCTCTCT CCG

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SEQ ID. No.: 6

FIGURE 24

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115-96 (3x0.01)

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1  GGGGAAATCTT TGAATGOCAT GGGGCTTTC GGAAGCTTGG ATGCTCTANA
21  ACGGGGCTCTT TTTTTTTTTT GTTTTCTGT TTAATGTTT TTAAAGAA
101 GGTTCATCA CACAGAACG CACACAGAT GGAAGAGCAT ANCTCTGCC
151 GACCTCTCA GCTGAGCTC TAGCAAAAC TTAGCTTTG TGTGCAAGG
201 GAGGCTGAG CCGGAAAGT GCAAGCTCT TGGCTCTTC CCCAGGCTC
251 CCGGCTGAG GAGTGGCAT GTATGAAGC CAGTATGAG CACTCTGCT
301 CTTGCTCTT CTGTGCTCA TTAGCAAGT AAGGCGGGA NOTTCTTGG
351 GAGCAAGG GGTTCAGAT GCGGAGTGG CATTAAAGG CTGAGGACT
401 GAGCAAGG TGTGCTCTT TGTGCTGAG AAGGCTCTT AATTGCTAC
451 TGGCTCTTG GGGCTGCTC GGAAGCTTGG GGTGAGATA AGTGGACTAT
501 AGGAGCTAT GGTGAAATT GCAATCTTG TCCCAAGCC NAGCTGCTAT
551 GGGAAATAT TAGGATCTT GGAACCAAC CCTATGCTT GAAACCTGT
601 TTTGAGGA NCTCATCTT GTTCCAGCC CCGGCGGGA GGGGCTTAA
651 TGTGCTCTT CCGTGGGGA AGGCGGGA GAGGCTTCT TGTGCGGA
701 AGGAAAGAG GGTTTTTTTT AAGGCGGCT CCGGCGGGA CCGGCTGCT
751 TGTGCTCTT TCGGCGGGA GGTGCTTCT GCGGCTTCT CCGGCTGCT
```

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FIGURE 24

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3949 P01 (50m)

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1  ggattccgg: tagyaatgaa attagaggt gtagattcca tggagccacg
51  gttaatttg: gtagccacag ttactcgaat tattcaccat ctcttgagga
101  tacattttga tggctgggaa caagagtatg accagtgggt agactgtgag
151  tccccgmo: totatccctgt aggg
```

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FIGURE 24

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scmg

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1  GGGGCTCAAG GAGGCGATC TTCCGGGTGG AGGGGGAAGC GGCCTGACTG
5  GAGTGGAAAT TTTTCCCAAC AACTTCTC GAGGCAACA TATTGGAAGG
10  GACTGGGAG GGGGCGATC CAAATGGAAG TGCTGAAAG AACTTCTCG
15  CCTGCTTAT TCTGAGCCC GGTGCTGCT GCGGCCCCC AATTACCTCA
20  TGGACTGTC GTCTGACAG AACATTCTT ACAATCCAA ANAGGAAGCA
25  NACTGTTTC GATGTTTGA CGCTATGAT AGCTGCACTG AGACACAAG
30  TAGGCTCTC AGCTCTGAG AAGTGAAGA AGAAGTCTT CTTTACCTT
35  CCAATCTCC AATCATCAAG AATAATCCAC AAGTCTACAC ATACCCAGAT
40  GGTAAATCT GATGCTTAC CTGTGAGAT TGTGGGATG TCCTGTTGG
45  AAATGCTTT TACTCTAAA CAAACCTTT CTGCACTTT TCCTGTTCA
50  GAATTAATC GTCCAACTT AAGAGAGAG CATTCTGCC NACTTCTAG
55  TTACTGCTT GCTCCCACT AAGAAAGCA AATGCTTCC AAGAGAGCA
60  TTAGTCTCT AATTGCTCT CTAAGCCCT NTCAGCTAC CTGCTGAT
65  CAGGCTGAC AAGAGAGAG AATGCTTCC TCTGCTGGA AAGGCTGCT
70  GGGGTTAC
```

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FIGURE 24

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C4-20 (Crick)

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1  TACGGCTGCG AAAAGACGAC AGAAGGCTTC GGGCTGGGAG AAGACGACAG
21  AAAGGAGGCG TTCTGCGGCT GGGGGGGGGG GGGGGGGGAG CTCTGCGGCG
101 GCGGTGCGGT TCAGTTTGGC GGGCTTCTGC TATATGCTGG GGTGCTGCT
151 CAGCGGCGCG CTCATCTTCT TGGCATCTG GCACATCATA NCTTTGATG
201 AGCTGAGGAC CGACTACGAG AAGCGCTATA GACCAATGCA ATACCGTGAA
251 CCTCTTCTGC TTCCAGAAAT AGTCATCCAC GGGTCTTCTT GTGTCATGTT
301 TCTCTGTCGG CAGAAATGCG TACCGCTGGG CCTCAATATG GCGCTTTTGG
351 CATTCC
```

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FIGURE 24

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C3-54 (referred to in p. 19)

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1 TACGCTGCG: AGAAGACGAC AGAAGGCGAG TGCTGCGCGG TCCAGCGTT
51 OCCATACCA: CGAGATGCGA TCTGCTAGCC GAGAGCCCGA ACACAGCGGA
101 CCGCTGCGA: TGTTTATGA CAGAAATGA GCGCGAAT ACCTTGGCA
151 CTCACGAT: ATTGACATCC AGACCAATAT GACTGAGCGA GCGCTGGAC
201 TCTCTGTTT ACCAGATGCT CAGCCTTCTT ACCTGTTAGA CATGCGCTG
251 GGTCTGCG: TGAGTGGAAA TATATCTCA GAGAAAGAC ACTACTGGT
301 GCGATGGA: ATCAGCGCTG CAGTGTGGA TGCGCGCTG GAGAGATAC
351 AGAAGCGA: TGCTGCTAG GAGTGGCGA AGCTCTCTT CAAAGCGCG

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FIGURE 24

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B4-14 (desig.)

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1  ACCGCTTCCA GAAAGCAGCA GAAAGGTACG OCTCCGAGAA GACGACAGAA
11  GCGTAC:GCT OCCAGCAGAC GACAGAAAGG TACCGCTCCG AGAAGACGAC
101 AGAAGCAGCA GAAAGCTCA AAAAGTATCC TCGCGAGCCA GCGCGAGACT
151 TGATTCACTT TCACTGCTC TCGCCACCCG CTCACCGCGG GTTTCGCGCC
201 GGAGGACAGT CAGATATGAA CGGACCGGTC GATTATTTAG TCACCGAGGA
251 AGAGATCAAC CTCACGAGAG GACCTTCGGG GCTCGGCTTC AACATCGTCI
301 GTCCGACAGA TCAACAGTAT GTCTCCAACG ACAATGOCAT CTACNTCAGC
351 COTATCAAAH AAGATCGGCG TCGCGCCCAH GATCGGCGCG TCACCGAGGG
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FIGURE 24

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1 AGCTTAGTC CCCAGAGATG TCCCACTAC TCTCTCTCT OCTGTGCTG
51 CTCTGGGQJ. ATTGAGGCC TTAGAGGCC AACTGATCC GTGTCCCTCT
101 TCAAGGATC CACCTTGAC ACAGATCTT AACCCACTG AATGATGCG
151 AACAGTGG: AGAGCTTTCT AGAGCTTCA CCTCTGCTG CAACCCCTCC
201 TTGTGCTC: TCTCAAGTT CATGACACC CAGTATTTG GAATATTGG
251 TTGAGGAGC CCTCTGAGA ATTTACGCT TGTCTTTCAC ACGGTTCTT
301 CCAACTTGT: GGTTCCTCC AGAGATGTC ATTTCTTCA TTGAGATGC
351 TGGTTTAC: ATGCTTTTA TCCAGGCC TCCAGCTCT TCAGGCCCA
401 TGGACCAA: TTGCTATTC AGTATGCGC CCGCCCTG AGCGGATCC
451 TTAGCCAG: CAATCTTACT ATCGGGGGA TCCAGATGC TTTTGTGAC
501 TTGCGAG: CTCTGTGGA CCCCAGCTG ATCTTTCTT TAGCCACTT
551 TGAAGGAT: CTGCGCTCG CTTCCCCAC TCTGCTGTG GCGGAGTTC
601 AGCTCTGCT GATGCGATG GTGAGCAAG GCTCTGGA GAAACCCCTC
651 TTCTCTTTT ACCTCAACG GATTCTGAA GGTCTGATG GCGGAGGCT
701 GGTCTGAG: GGTCAAGCC GCGCTACTA CTTACCTCC CTCACCTTA
751 TACCACTAC CATCCCTCC TACTGCGAG TCCAGATGA GATGTGAG
801 GTGCGGAG: GGTGAGGCT CTGTGCGAG GGTGCACTG CCATCTTGA
851 CAGAGGACA TCCCTCATC CAGAGCTAG TCAAGGATC CCGGCTTGA
901 ATAGAGCAT TCGCGATAT GCTTCTTGA ATGCGAGTA CTTCATTAG
951 TGTTCAGAG CCGCAAGCT TCCCTCTCT TCTTCCACC TTGTTGAGT
1001 CTGTTTAA: CTCACAGGC AGACTATGT CATCAAGAT CTTCAGAGC
1051 ATGTTGCT: CTGCTGTTG GGTTCAGAG CTTGATAT CCGCAAGCT
1101 GCGGAGCT: TCTGATCTT TCGGAGCTC TTTTGGGCG CCTATGTGCG
1151 TGTCTTGA: CTTGCGGAG AAAAGCTCG CCGCGCTG GACTGGGCG
1201 GTCTCACT: TGTTCAGAG CAGCGGCGG AAGAGGAG TACGAGGCG
1251 CAGTTCTTA AAGAGGCGG TGTTAGGCT ACAAGCTAC CCGGCGAG
1301 CAGTATGCT TCTTTGAT TAAAGAACT AAAAAAAAA AAAAAAAAA

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FIGURE 24

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1 TCTTGGAAAG CCGGCTGAG GCGAATCTC GACAGGGAA TCTTGGAGT
51 GGAGATCTT AATCATGCT GTTCCCGAG AGCTGATGA GATCCACAAG
101 GACCTTCAGA ACTACTATG GAATGTACTG AAGACATCTG CAGACCTCCA
151 GACTAATGCT TGTGTCAAGC GAGCCAAAGC GGTCCCGAGC TACATCCCGG
201 AAATCTTCA GAATGTACAC GAAGACCTTA GTTGGAGCTA TTATGGCTGT
251 GGTCTGACTG TTCTGAGCG GCTGGAAAAC TGCCGAATTT TGGATCTGGG
301 TACTGGAGCT GCGAGGATG CTTATGTCT TAGCCAGCTG GTTGGTGAAG
351 AAGGACATGT CAGCGAATA GACATGACTG AGTCCAGGT CGAAGTGGCT
401 AAACTTATC TTGAACACCA CATCGAAAA TTGGTTTCC AGCCACCCA
451 TGTGACTTTT CTCCAGGCG CACTCGAGAA GTTGGCAGAG GCTGGGATCC
501 AGAGTGAGAG CTATGGTATT CTCATATCCA ACTGTGTTAT CAACCTTGT
551 CCTGATGAA AACAACTCT CAGGAGGTC TATCGAATGC TGAAGCACGG
601 CGCGGAGCTC TATTTCAATG ACCTCTATGC CAGCTTGAAG GTCCAGAGAG
651 ACATCAATGC CCACAAAGTT CTATGGCGG AATGCTGAG AGGCGCTCTG
701 TACTGGAGAG ATCTTGGCAT CATGCCCAAG AAGATGGGT TCTCCCTCC
751 ACGTTTGGTC ACTGCGATA CTATTACTGT TGAAGACAAG GAGCTCGAAG
801 GGGTTCTTGG TGAATGTGG TTGTGTCTG CCACATTTCC CTTCTTCAA
851 CTCTCTAGA CAGAGCCAGC GAAAGATGC CGAGTTTCTT ACAATGGAGG
901 AATCAAGGA CATGAAAGG AACTAATTTT CGATGCAAT TTCACATTC
951 AAGAGAGGA AGCTTTTGA GTTGATGAG AGACCGCAGC TGTCTGAAG
1001 AACTCAATG TTCTCTGGA TTCTCTCTC ACACCTTGTG AGGCTCTGCT
1051 GCGAGCTCC CAGGCGGCTT TGTGAGTTAG AGACAAAGT TCTAATCAGA
1101 CATCAATCA AGCTTGCAGA GACTCTGAC AAGATGAAGC CCAGACATGC
1151 ACCTGAAGCC ACCGAGGCT GCTGTGGCA GAGGAAAAC TCTAGATCT
1201 ACAGCCAGCG CCGAGCCAC CGGCTCAAG AGGCTGCTA AAGGACATC
1251 ACAGAGCTT CTTAGCTGC TCTTGGCAG TGCACAGATT ATGTGAAGT
1301 GCGAAAGCA CCACAGCTA GACCACTCT AAGAATAAGA GTGACTTTTA
1351 GAGATTTTA ATTGAAGTT CACAGCAAT CGCTCTCTT TCTATTTCT
1401 TATTTCAAG TTCTGCTCC ACCTAGTGT CAGAAATAGA ACTTGAAGC
1451 CCAAGTTTA CTCAAAGGCG AAGGACATC ATCAACCTG TGAAGATTAT
1501 CTCTCTCTG GCTTACCACA GACACCTCT GGTCTCTCT CTGTGGTTAC
1551 CAGTAAGCTC AATCTTACT AATTTATGC TAACCATGAC AAGATTTGT

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1601 CAGCTAAT TTCTAGAG TATTCTTAG GTTCTCTCT GCAATTTT
1651 TCTCTTAC TGAATGAAA AAAAAA TAAATATA ATTGACTT
1701 GAAAAAA. AA

SEQ ID. No: 14 cont'd

FIGURE 24

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1 CCGACCCCTG GCGGAGTCTG TCGAGCCCCC GGTCAAAGAC GGCATCCTCT
 51 ACCAACAOCJ COTAAAGTTT GZCAGAAAT GCTGCTCCTA AGTGTGCGCT
 101 CTGCTGTATC CCGAGGCCCC ATCAGGGGTA GCTGCTCTAG AAGCTGGA
 151 COTGCTGA! GGTGCTCTG GACCAACAGG CGACAGCTCC ACAGGCCCCA
 201 CCGCTCAGX! GGAACGCCCC GTCAATAGCT TGGCTGACTG TGTATCTGTC
 251 CTGCTGCTX! ATGCGAGAG CTGTCCGAGG GACACTGCTG CTTCTCTGAT
 301 TACCAGCACT! GAGCGAAGCC ACCTGTCTGC TGCACAGCAC CCGCAGCTCT
 351 GGGTGAACCT CATCTGTCTG CTGCGCTTCC CGGCTACCCG AGAATGTTG
 401 TCAAGATCTG GACAGGCTGA GALTCCAAA AGGCGCTTTC TTCCCATGGA
 451 AGAAATCTCT ATCTACTCTT CCTGCGAGGA AGTGAACGAA TTCTCGTGA
 501 TCGTGCAGAA GACAGAAAGC ACCTGCTCTT GCGAGCTGAA AGGACCTTAC
 551 CTCTCTCTG: TGGCGCAGA TGACATCCAA CTGAGGAGA CATCCAGCC
 601 CCAAGCCCTG! TTTAGCTGCG CTTACCGTTT CCTGCGCAG TACCGCTCTG
 651 ACAAGGCTGT GTTCTCTGTT GAGGCTGCGC GCGCTGTGA CTCAGGTGAG
 701 GCGCTTCTTJ CTTCTAGTAG CCGCGCTGCC CCAGACATAT CTGGGCTTGT
 751 GCGTCCCGG: ATTGCGCGG AGCGGAGCG TCTTCCAGAG CTGGCATGT
 801 CCGCAGCTTJ CCGCTGCTT CCGGCGCTCT CCGTCCCTC CCTAGAGCCC
 851 CCTCGAGAG: TTGCGGAGGT GCGGCGAGGA TTTGAGCTCC CCACTCCAG
 901 AAGCTTCTT CTACTGATC CCGGCGCTCA AAGCTAGCA TTCTCTCTA
 951 GCGGCGAGC: AAGAGGAGC GGCATCCGCT CTCTATGCTT CCGTCTGAA
 1001 GCACAGCAGC AAGCAGAGG GCAGGCGCA GCATTTCTAT GAGAAGCTGT
 1051 GCATCTGGA GCGCAGCTT GCGCTGACCA ATGCGGCTCC TGAAGCCAA
 1101 GAGGCGCTCC TTGCTGCGG GAGGCGCTT GCGCAGCTT ATCTACCATA
 1151 AACTGAGGA TCTGATTTG CCGGCTCTG CCGAGGAGC CAATCTGGA
 1201 GCGCAGTACC GAGGCTGCT GGAAGTGAAG CTGGATGAAG CCGAAGCGC
 1251 CCGCTGCTT GAGGCGAGG GAGGATCAA GCGCAGCTG GTGACCTGCT
 1301 TGAAGCTTA ACGAAGAGG GCGGCGGCG CCGTGTGAGG GCGCTGAGG
 1351 CCGTAGTGC CAGCAGCTG AGGACAGAG TGATCAGCA AGACAGGAA
 1401 CAGTTCTGA CATAGGCTT CTACTCTGAC CTGAGGAGC AAGCAGGTJ
 1451 GCGGCGGAG GAGGCACT CTGCTTACC TCGCTCTCA GACTGTACG
 1501 ATTGAACAT AATAAGCTT CCTATCAAC TTCAAAAAA AAAAAAA

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FIGURE 24

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1 AGAGACAGG TATCCCGCC CTCCACCGG GCACTTTTA CTCTAGGG
 21 AAGAACTCC CAAATCCAT GAACTCTGA GACTCTCTA AGCTCCCTA
 101 GGAACCTA GCTTTTGA (GCACTTCC CACCGAAGC TAACCGGTA
 121 GCGGAGCC CACTCGATT GATTCACAG CTCCTAGAC AGCTCAGC
 201 TAGCTCTG GACCTCGGA GAGGAGAG ACATTAGAG CAAAGCTGT
 221 TAACAGAGT GCTCCCTAG GCTCGAGG AGAGCTCTC TTTTCACT
 301 GCGGAGGA GCTCGAGTA GAATGCTGT GCACTCTG CCGCAGATG
 321 TCTACTCT ACTGCTCT TTTCTCTG TCGAGCTC ACACAGTGG
 401 AGCCCTCCAG AGCTTCTC CTCTCTCTC GCGCGACT AGCTCAGC
 421 GAGCAGCTC CACTATATC AATCTTGA GCTCAGTC TTTCTCTGA
 501 ACACAGAGG GACCTCAGC ATTCTGCGC GCTCTCTG AGCCCTAGT
 521 ATTCTCTCT TCTCCAGC GCTAGAGG CTCTATCT TCTCTCTA
 601 CTGAGTCTC CACTCTGA GACTCCTT GCGCTATGG AGCATGCT
 621 AGCTCTCTG TAGCCAGCC TCAAGCTC TCTCTCTA GAAACAGAG
 701 GAGGCTCTA AGCAGGAGC GCGCTGATC GCACTCTG TCACTCTG
 721 GCAATCTCC CAGACAGCA AGCTCTCTG GCTCTGAGC TCTCTCTC
 801 GCTCTCAGC TCTCTCAGC AGCTCTCTC AGATCTCT TGTGAGATG
 821 TGTATCTCA AGAGGAGT GCACTCTT AGCAGTACC TCGACCTC
 901 TCAAAAGT GCAAGCGG GCACTCTG GCTCTCTG CAGCAGTAC
 921 AGAGCTCTA GCAAGCTG AGCTCTCTG GCTCTCTG AGACAGTCA
 1001 TCTTTTAG AGAGCGGCT GAATCTCA GTCTGAGAG TCCACCTAG
 1021 AGCTCTCTA GAGATCTG ATATCTCTC CAGAGAGAG GAGGAGAG
 1101 GCTCTCTA GCACTCTG GCTCTCTC GCGCGGCT ATTCTAGAG
 1121 AGCTCTCTC GCTCTCTG TCACTCTAG AGCTCTCTG TAGTAGCTT
 1201 CAGAGCTCA GCTCTCTC AGAGAGAG TCTAGCTCA GCTCTCTG
 1221 AGAGCTCTT GCTCTCTG TCACTCTA CCAAGTCTC CAGCTCTA
 1301 GATCTCTG TACTCTCTT CAGCTCTG GCTCTCTA AAAATCTG
 1321 TCTCTCTG GCTCTCTG TCACTCTC GCTCTCTG AGCAGCTCA
 1401 GCTCTCTG TCACTCTC TCACTCTA GCTCTCTG AGCAGCTCA
 1421 TCTCTCTA AGCAGCTC TACTCTCT GCTCTCTG TCTCTCTA
 1501 AGAGCTCTA GCTCTCTA AGCAGCTC CAGCTCTG TCTCTCTG
 1521 GCTCTCTA GCTCTCTG GCTCTCTG TCTCTCTG TCTCTCTG

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FIGURE 24

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1601 TCTTCCAAGG GAAATCAGC TAACTACACC ATCAAAGTCC ACATGAACCT
 1651 OCTGTCCACT GTCTTCTCTC TAAAGCTGAG CTTCCTGCTC AGCAGGCTG
 1701 TGCACTTAC: GGGCTCCGAA GCAAGCTGTC GCACCACTGC CATGTTCCTG
 1751 CACTTCTCTC: TCTTTCCTG CTTCTCTCTG ATGAGGCTCG AGGCTTACAA
 1801 TCTCTACCGA CTGTGTGTGG AAGTCTTGGG TACTTATGTG CCGGCTATC
 1851 TCTTAAAGC: GAGCATGCTG GCTGAGGCTT TTCTGTCTTT CTTGTCACT
 1901 CTGTGTGCT: TGTGTGATGT GAATACTAC GCGCCATTG TCTTACTGT
 1951 GCGGCTGAG: CCGGAGCTG TCAGCTACCC CTCTATGTGC TGAATCCCG
 2001 ACTGCTGTG: GAGCTATGTC ACCAAGCTGC GCTCTTTCAG TCTGTGTTC
 2051 CTGTCTTAC: TGTCTATGCT GCGCAGCATG GTGTGTCAGA TCTTCCGCT
 2101 TCGCCCTCAG: AGCAGAACT GCGCCAGCT GCTGACCTG CTGAGCTCA
 2151 GCGTGTGCT: TGGCTGCTG TGGCTGTGG TCTCTTTTC CTTTCTTCT
 2201 GCGAGCTGT: AGCTTGTGAT CCTGTACTTC TTGAGATCA TAACTTCTA
 2251 GCAAGGCTT: CTGATCTTCT TGTGTACTG GTCCATGCG TTCCAGGCCC
 2301 AAGCGGCTG: CTGCTGTGT AAGAACAACT CAGACAGCC CAACTCTCC
 2351 ATCTGTCTG: GAGGAGCTTC CTCCAGGCGG ATCTAAGCCA CCGCCAGCC
 2401 TCGCTGTCT: GAGGACACAT GCATGGCTTC GCTCAGGAT GTCTGTGCG
 2451 CAGTGTGTG: CCGAGCCAGC GTTGTGTGT TATGTGATA CTAGAGAGG
 2501 GCGTGTGCT: TGAAGGCTA GCGCTGTTC TCTGTAGGT AGATACCTAG
 2551 GTTCTCTGT: GAGGAGCTTC GTCTCTCAA GCGCTCAGAA GCACACTGCC
 2601 ATCTGTGTT: TGGGCGGCTT TCACTCTGGA CTTAAGGCTT TGTCTTCTG
 2651 GCGCTGTG: GCTCAGCTG TTCTCTCTGG GTTGTGAGAC CTGAGAGCC
 2701 AAGTGTGCT: TAAATCTGGA AGGAGGCTGA CACATCCGG CTGAGACAG
 2751 GCTTACTGT: TTAATTTCT GTCTGTCTTC TGTGTGACC ATGAGATCC
 2801 GGAAGGCTG: ACTGGGCTA AATGTTCTGG GAGAGGTTT GAGGAGAGG
 2851 CAGCTTACGA CTGAGCATC TCGCCAGCC TTCTGTCAA CCGTCTCTT
 2901 CATCTCCCT: CCGCAAGCC TCTCTGTGT TCCCTAACC CTCCAGCTGA
 2951 AGCTGTGCT: CTTAGACCA TCTGTGATT TGGGCTGTA GTTCCAGCA
 3001 GTTCTCTGT: GCGAGCTAT AACTCTCTTC TGTGTGTGG CTTTGGGCT
 3051 CTGACTCAG: GAGGTTTCT GTCTGAGGCC TCTCTCAGG CTGCTCACC
 3101 TTTCTCTCA: CTTGAGAGG ACTTCACTT CTCTGAGG CTCTCTCTC

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FIGURE 24

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3151 TGGCAAGTA: TGGCATAAG CCACCCCTTC AACCCAGCAC TCTGAAGACC
3201 AAGACAGCC: CCTCTGGTGA CACTGGCCAA GCTTGATCTT TTTCCTAAGA
3251 AGTGTCTT: AGATCCGCC AGGTCCCTCA GAAGACACTG GGTGCTTAG
3301 TGTGAATTC: GTCTACTAA COTACAGTGA GCACTCTCTC ACCCCCAACC
3351 CCGCAAAAG: TCTCAACAC TCTTGAAGTG TCAAGCAGGG GGTGGAAT
3401 CCAGGAGGA: TTCTGCAAA AGGCAGCAT TCACTTTGAC CTCAGCCCTC
3451 AGGTTCGGG: GAATTTTCTT TTTAAATACC AGTTCATTG TCTTTTGATA
3501 TTAAAGCTC: TTATAAGAG TCTGAAACT GTAAGCAT TCTGAGAGA
3551 GAAATAAA: TGAAGTCTTA TCTATGCTA TGGCAAGCA GCACAAAA

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FIGURE 24

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AFTP2

BMP1 TGF β 1 PDA

Seq Length: 430 February 24, 1997 13:27 Type: N Check: 1795 ..

1 AAGGACATGG CCGGCCCCGC GCTGCAGGCC CAGGCTCCAN GCGGCTCTCA
 51 ACTGAGGTTT CTACTGTTTC TGCTGCTGTT GCTGCTGCTG CTGTCATGGC
 101 CATGCGCAGG GCGGCGCTG GCAATGCTG AACAGCGAGC CTCGCGCCCT
 151 GAGTCCCAAC TCACGCGCGA CAACTACCG GGTGCTTTC AGGACCTGCT
 201 GAGCGCGCTG CATGCGCAGC ATAGCGGAGA GCACTCTAAC TCTGAACCA
 251 GTCTGACCA TCTGCTGGA TACTGANTCC ACGGTGAGA TTGCGGTCCA
 301 CGGCGAGCTG CTACTGCTG TCAGCGCGGC GTCGCTGANT CAGGCTCTCC
 351 CCGAACCTAC CAGGTGAGC AGCGCTGCT GTCGCTGAGC AGCAAGCGCG

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FIGURE 24

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D77 - TAB4/PE4

1 CGGACGCTG GCGAAGCCG GTGGGATAT ACOTGTCTG GTGCGCTACA
51 ATTGACACG CTTCTTGGG GGGTGGACCA CTTGCACTG TAGTCTGACT
101 TCTAATGAG ATGTGACTAT CACTCAATA ACCTGGATGA AGAANGATTC
151 AGGTGGATCC CAGCTCTTG TGGCTGTCTT CCACTCCCAAG AAGGGGCCCA
201 ACATCAAGA GCGAGAGAG GTGAAATTCT TGGCTGCCCA ACAGGATCTG
251 AGGAACGCAT CTCTGCCAT CTCGAACTTA AGTGTMAAG ACQAANGCAT
301 CTATGAATGT CAGATTGCA CATTCCCGAG AAGCAATANA AGCACCATG
351 CCTGCTGAA HGTGCAAGCC CGACCTHANA AACTOCANA AGCCTTGAC

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FIGURE 24

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1 C3AATTCCTG GGTGACCCA CCGGTCCAG AGGTAGACA GAAAGCCCG
 51 AAAGGCTGT GAGGTCAAT GAGCCCATG GAACTGACA ACGTCAGAT
 101 CGAACCCTG GAGGACAGT GAGCCCGGA CAGTATTCAG GACAGCTACA
 151 CCGCCATOGA AAICTCCGAC AAGGACCCCA TGAACAOTCA ATTGCTAAT
 201 GAAQATOGCG AAATCAGAA GTTCCTGACA AATGCTTTT TAQCGAAGAA
 251 GAAGCTAGCC GATTACGCG A.TGAGCATC ACCCTGGAAT GACTTCCTTT
 301 GGAATGTCTT CATTTAAGCT GAGGACGCG ATCATOGGCA GTGGATCTT
 351 AGGCTTGTTC TAGGCCATG CCAACACCG GATCATCTTT TTTATAATCA
 401 TGTGCTTAC TGTGGCAATA GTTCCTCTT ACTGCTTCA CTTTGTCTG
 451 AAGACAGCCA AGGAGGAGG GTCTCTAATC TATGAAAT TGGCGAGAA
 501 AGCATTTGGA TGCCCTCGGA AAATTGAGG GTTCATCTCT ATTACATCT
 551 AGACATTTG AGCCATGTCA AGCTACCTCT TCATCATTAA GTACGACTG
 601 GCTGAGTTAA TCAGAGCTT CATGGACTT GAAAGAAACA CTGGGAAATG
 651 GTACCTCAAC GCGAAGTACC TCGTCTTATT TGTGTCCCTG GCGATCATCC
 701 TCCCGCTCTC TCTCTTAAA AATTGAGCT ACCTTGGCTA CACCAGTGA
 751 TTTCTCTCT CTTGATGCT GTTTTCTCT AGTGTGTTA TTTACAAAA
 801 ATTCCAAAT CTTGCTCTC TCGCTCTCT GATCACAAC AACGGAATC
 851 GACGTTTAA GACACACTT CCGATTCACA TGATCTCTCT GCGTAATGAC
 901 TCGAGAGCT CAGGTGTGA CTTGATGAT GATTACGCTC ACCACAACCC
 951 AGCTGGGTT GATGAGAGG AGGTGGAGG CCTCTTTCAC AGCAATGGCG
 1001 TCGAGTAGA AGCCAGGCT GCTGAGAAAT GCGAACCATA ATACTTTGTG
 1051 TTCAATTCCC GAGGCGCTA TCGAATCCCA ATCTGCTT TCGTTTTGT
 1101 CTGCCACCTT GAGGTCTTC CCACTACAG CGAGCTTAAA GATCGATCCC
 1151 GCAQAAGAT GAGAGAGTG TCGAATTT CCACTGAGG CATGCTCTC
 1201 ATGTACCTT TCGGCGCTT CTTTGGTTAT CTGAGCTTCT ACGGCGAGCT
 1251 TGAAGACGA CTTGCTCAT CTTACAGCA GGTCTACACA TTTGATACCG
 1301 CTCTTCTCA GGTGCGCTG GCACTCTCG TCGAGTGAAC ACATGAGCG
 1351 AGGCGCCAT GGTGCTTTC CAGATCTCT ACTTCGTTAG EATCAGACTA
 1401 GGTGCTTTC AAGGAAACCC TTTGAGGCG TGAAGCATTT CAGGATCTC
 1451 GGTGCTTTC TCACTGACT CAACAAATC CTGCTCATCC TCGTCTCTAC
 1501 CATCAATTA ATCTTGGAT TCAAGGCGC TCTTCTGCC ACTATGCTGA
 1551 TTTCAATCT TCGGCTGCG TTTATCTCA AGCTCTCAA GAAAGAACCT
 1601 TTAAGATCAC CCGAAGAT TCGGCTTTG GTCTTCTTG TCACTGGAAT

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FIGURE 24

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AFTP1

1651 TATTTTCANR ATGGGAACA TGGCGTCAT TATACTGAC TCGATCTACA
1701 ACCCGCCGAA TCCTCAATCAC CACTAATCCC GGGGAGACGC GTCTCCACTG
1751 GAAACAAGTG AATTGTCTG AAGGACATTT TAATTGTCTT GATTGGCATG
1801 TTAGTCTGAG GATTAGCAA GATTCCAAAG ACCTTTTCTT AGCTCTATCA
1851 GCACACATTT TACCCGAGC COTOCAGTGC AGTGTGTGAT GCGCGAGTTC
1901 TGTTCGAGC AGTGTGCAA GCTGAAGCCT GTTCGCTGCG TGTGTGCTC
1951 AGCAGACAAT AGCTGTCCC CCCATGCTCA CTCCACTTCT CTCCACCCC
2001 AGATTAACAG GTATTCTAC TCTCAACA TCAGACAAAG ACCTCTCTGT
2051 TCGATACTT GTGGAAGAGA AATTATGGG TTTTGTTCG AATGTTTTG
2101 TTGGGAATGG TGAAGATGC ATTAAJAATT CTGTGACACA CATTTTAACC
2151 CAGGCGCTGC AGTGCAGTGT TTGATGCCC AGTTGTOTTT GAGGAGCTG
2201 TCGAAGCTGA AGCTGTTCG CTGCTGTGT TGTGAGCAG ACAATAGCCT
2251 GTCCCGCCAT GGTACTCCA CTCTCTCCA CCCCCAGATT AACAGGTAAT
2301 TCTACTCTCA GACATCAGA CAATACCTC CTGCTTGGC AGTATCATC
2351 AATTACGCGC ATCTCTACT CTACACCAAC ACTAAGGTC GGTGACTAG
2401 CTGAGCGAGG GGATATCTT GGGCTGTCCC TGTGAGGATC ATGACGTATG
2451 ACGGTTGICA GTATAGAGTA CTTCATTCA ATACTCAAGG AATAGTTTGC
2501 CCAACCTTCT TTTACACCG AGTTAGTGA

SEQ ID. No: 19 (cont'd)

FIGURE 24

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1 GTACATATCT GTCCGGAATT CTCCCGTCTA CCCAGCCTC CCACCGCTOC
 51 CAGAGATCA CAGAGCGCG CCGTCACTCA GACCCAGAC TCCCGCTCC
 101 ACCGAGAG CAGAGAGAC GCGGACCTT CCGCTCTAG CCTGATCCCT
 151 GTCTTGGCC CCTGACATT GCGAGCTGA GAGATCGCT TCGTAGAG
 201 TCGATCGTA CTCCCGAGA GACCATCTT GAGCGCTG AAGAGGATT
 251 GGTTCGACCT GTTTCAGAC GTTCACCTA TCTACTAGA TATCAGACT
 301 CCGCAGACA TACAGATAA GTTCACATG CCGTCTGCT GCATCATAT
 351 CCGCAGCGC CATGATGCC GTGACATCA CCGTCCAGT GCGAGCCCA
 401 GAGATCTCT CCGCAGATC GTTTCGAG AGCGAGAGC CATCAGTCT
 451 TGTGAGAGA GCACAGACA TTCCCTGCA TCGAGTTTA CACTCAGCA
 501 TTCCAGACG AACACAGCTT AGGTGCTTC AGCAATCTC TCTGAGAGA
 551 CTGAGTGGC CCGTCCCGC CCGCTCTAG CAGCTATGC TACACGACC
 601 CCTGAGCTC AGCGCTATG TCCATACAG GCGCGTACC CCGCAGGAC
 651 TCAAGTTCT TATCCCGCA AGCGCAGGC ATATCAGTG CCATACAGT
 701 ACCCGTATG AGGAGTTAT GACACAGGC CTCCCAACA AGTCATCAT
 751 CCGCAGCGT ACCGAGACA TACAGTGC CCGCTCTAG GCATCTGCG
 801 CCGGCGAGC ACCCGCATG CCGTGGCTC TCTGTTCTG GTCTTCTAG
 851 GCCTTCAAC TTTCTCTGC ATAGCTCTG TTAGTCTGT GTGAGTAAT
 901 TTGATTTGA GCGATTTCT GTTTGTGCA AGTGTCTTC ATAATAATT
 951 AATAGTCTT TTGAGGCTG GTAATCTAAT AATTGTGCT GACCTGCAT
 1001 GTACACAAA GAAAGCCCGA GGTATCTGT GAGTGAAGC CTGATCTCT
 1051 CCGGTAATA GTTTCACCA AGTCTTTCTT AGCGACTTTT GATGCGTTT
 1101 ATGTAAACA ACCAGTTAA ATCGGCAAT TCCGTCCAGT TAGGTGAGT
 1151 GTTGAATTA GCGATGCTT TCGTCTGAT GCGAATACTA ATACTGCTA
 1201 TCGAGGAGA TGTGTGCA TGTGTGAG AGAGTCAGC CTCTTTAAC
 1251 TGTGATCTC CTCTAGACC CCGTCTGCT GTTACCTAG GAGCTGTGG
 1301 CTGCTGCTC CTGAGACT ATGCTGTAG GACCGTTAA CGTACCTCT
 1351 GAGCATTAA GTTACCGTT GAGCTCTTA GGTATCACA GCGGATTGG
 1401 CTGCTCTGA TCGAGAGGC CAGCCCTCC CTTTAAAAAT TACGCTCCG
 1451 TAACTCTCC AGTTTAAATT TGTGTTAAT CTCTGTTTG CTCTCTCTG
 1501 GGTGATGTA CATGATCTG GAGTTAGGA TTGATTGAG TCCCGTGGG
 1551 TCGAGGATG CAGGAGCTG TCCAGGACC TCGTCTCAT GTTGTTTTA

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FIGURE 24

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1601 GGTATATTCG. TTCTACCTC AGCTGCAG GAGCTATAG AGACTATTTT
 1651 TCTACTTTGT. AAGAAAGTAT GTTCAGGGGA ATTGAGGAGA GCCTTGTTTG
 1701 AATGTTCTCT. CCTCAGGCTT CCTGCGCGCC ACACTGCGTG GTCTGCGGA
 1751 GCTTFTTCTT. CAGCAATGGA AGCCAGCGCC GTGCGCTGTC CAGATCTCA
 1801 GGTFTTCACT. GAGAAATGCG ATTGCGTAGA GCATCTCAGG GATTGTTCTT
 1851 AATGCTCTAC. GTTATGCGGA TCCAGCTGTG TTCTCACTCC AGACCGCTTC
 1901 AGCTCAGAA. AGCTTAAAC ATTTCTGCTC CCGAAATGTG TGCACTCTG
 1951 AGAAGCTCA. AATCTGCTT TCTAACGAA ATTTGTATTT CTAAATTAG
 2001 AGAATCAAT. TTCCAGCAT TTAATTTA TGTCTTTCA TGTTFEAAAG
 2051 CTGCAAAAT. CAGCTTTGTG ACTGCCATAT TTTAGTTTCA AACAGTACCC
 2101 CCGCAGAA. CTGGATGCG ACAGTGAAG CCGCGCGCC TCTACTTTG
 2151 ATAGTCTGT. TTCTCCAGG TGCTCCAGG AAGCATTCAT TGTGACTTTG
 2201 CTCAGCGCA. TGATGCGTG CTGCTTGGC GCGTCTGTC TGCTAGCTC
 2251 TTCTTCTGT. AGATCAAGTC TCAACAGAT CTCCATGTA CACAGTTGCC
 2301 AAGTAGATGA. GGTGTTGCG ATAGTCTTT CTGATACTC CTGGGGACC
 2351 TGTTCAGACC. TGCCATTTC CAGCTGACAT TTGTTTTCT GTCATCTCTG
 2401 ATAGATXGA. TATGTGACA CATGOTACG ACOCCTTCA GTGCGCTTT
 2451 AATAAGCAT. ATGCTGATTT CACATCTGT GCTGTATGAC TGCCATTGCG
 2501 TCACAGTGT. ACCATTGCTA AAGCTCCGTG CTTTACTTAC AAAACACTAA
 2551 AACAGTGTG. TACTGTTTCA CAGTGATTTT AATTTTAGAG TTAGTACTG
 2601 GCAITCTTA. AGCCATAGAG TACTGAGTCA CATCCCTGA GACTTTTGA
 2651 AACAGATTG. TCTGCTACTG TCCCATGCT GTGCGCTGCG TGTCTCTGG
 2701 TCCCAATGG. CTTAGCTGTA CAGGCAAGC ATAGTTGAGC CTGATCATTC
 2751 CTGCAAGAG. TTTGACTTGA TTATATAGC AGAATGGAAT ACATTCTTGG
 2801 GCACTCTAGT. TCTCAGCGCC TGATCTCTAT AGACCGCACC CTTTCAATG
 2851 CTTTGTGCG. GTCACTGTA CTTCACTGAG TCTCCGATT CTTGAGTTAG
 2901 AAGAGAGAG. TTCTCAGCA TTGAGGAACA TGTCTCTCC ACTGAGACTT
 2951 AATGATGAT. GCAAGGCTGG AAGAGCTGG CTGCTGACAC TGCATCTGCG
 3001 CTGATGCTAT. TGTCTCTTA TTCTTTGAT TTAAGAACCT TTCATATGGA
 3051 AAGCTGAGG. CTGCTGAGA TGTCTCTTG CCAAGAGGG CTGCTTAAAG
 3101 TCAATGATK. CCACAGTAC CTTCTGAGT GTAGCAAGTT CTTGCTTTG

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FIGURE 24

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3151 AGACAGAATC GTTCAGATTT ATTTCTACA TCTGTTTTO ACCCCATOCA
3201 CCTCTCATTT TTCCCTTCCA GTCTACOTAG ATGAAGATG AAGGCCAGAG
3251 GATGAGACAG GTCTTCTTTG TAACTCTTC TTTTATTCTG TTGCATCTAC
3301 CAGGCCCTTT TTCTCCCTGT CTCTOCATAC AGTATOTTTA TAACTGAAC
3351 TGTAAATAA TTAATGATC ACTACCTTA AAAAAAAAA AAAAAAAAA
3401 AAAAAAA

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FIGURE 24

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1 TACCGGTCCG GAAATCCCGG CTCGATCCAC GCGTCCCGGG CTCCGAGAAG
 51 ACCGACATTA CGAGGCCAAG CCGAGGCAGG CAGGCCACTGC AGGCACTGAG
 101 AGCCTTTCAG TCAGAAAGCA TCCACAGACA CGAAGAGGCC GTTAGGAGAA
 151 AAGCAACAA ACTGAGACAA CCAATGAGA TCCCTGAGGA GTCTGAGCA
 201 AGCCTTAAAG TGTCCCTAAA AGACATCACT GAAATAATGG AGAGACATCT
 251 CAGTCATAT GAGGGAAGCC TGTCTCAGAG TCAAAAGCTC TCAGATGAG
 301 AGCTGATTA ATGAGCATCT GAGAGGCTGG TCTGCAAGG AATTATAG
 351 ACCAGATACC CAGAAAGCCT GATTCAGAG AGAGAGAGAC TACTCATCT
 401 CCTTATACAG TTCTACTTTC CTGAGCCAGT AGTGGGACA GAGTAAAG
 451 CAAAGGATTT TTCTCTTTT CAGGACAGG CCATGTTTAC ACAGACTATA
 501 GAGAGGTTGG GTTTCAGTTC AAGCCCTTCA GTTAAAGGAG AAGTTGCGG
 551 ACTTAGTTTA GAGCTGCTA TGGGTACAA CAGACAGACA GAATCTGAG
 601 ATAGCTACCA GTCCATTTCA AAGCAAACTT ATTTTCTCTC AGCCAGGTTT
 651 AGCTACATCC CATGAGCCAC CTGCAATTTT CAGTCAATG ATCTTGAAT
 701 CTGCAAGCTT GCTCTCCAGG AACTTAAAG TATTGAGAA ATCTGAGG
 751 AGACTACACA CAGCCAGAT GCACTACCTT TACTGAGCA CAGGCTAAA
 801 AACTTTTTC CAGGTTTGG GTCTCATGCT AACCAGGCTT CTGTGACCT
 851 GCGGAGATC TACTGCTGGA AAGCCATTTT AGAAGGTTTC AAAAGTGAG
 901 ACTGAGTGA TGTAAAGCAG CAGGAGAGG AGTCTTTGAA TATTACATT
 951 ATGAGCAGTT ATGAGGCTT TGAAGTTAAA GTTGTGCGA GTTAAATAT
 1001 AACAAATTA AATCAAAAA CAGCATTTTA CAGTAAACT CATCTAACT
 1051 CGCAAACTA GTTACAACTA TCTGTAGCCA AGATAAGTGG ACCAGCAGAA
 1101 GCAATAGCA TTGCCCAGTG CAGAGCTGGC CTTGTAGCTA GCAATCAAC
 1151 CTGATCTGTT ATTGATAGCA AACTGCAATT GTTACCTATT TCGGACATTA
 1201 TCTGTCTGAG TCACAGAACT GAATTTAAGA ATGCTCTTCA ACTGCTAAC
 1251 TCGTCAAAAG ACCACTACAC TCGTCTGACT GAACTAGCTG CCCAGATTCA
 1301 AGAAGGAGCA GAATTTCTGA CTGCTAGAAA AGAAGCTAAG CTTTCTCTAA
 1351 AGATGCTGA AGCTGAGAG GTTCTGATC CTGAGGACA CCTTAAAGAG
 1401 TTATTAAGAT TTATGCAAC ATTGAGTCAA AAAATAAAAA GTTATGACAT
 1451 TTGATTAAC ACATGCTCA TAGATTGGA TGTCAAAAT TTCTAATAA
 1501 AATTTGCTA CTCTGCAAA AATTCACCCA GTTATAAAC TCACTTTATT

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FIGURE 24

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1551 AAATCTKAGT TOTGAGGCT TCTAGAACCT CATGTCTACA AAGTGACAA
1601 CTTTCTCTCA GCACAAATCCA TCATACAGTG GATCAATCAG TCAGAGTCAG
1651 AAGAACTACT AGTCAAAATT AGCTCATTTT CTGAATTCAT TAACACCTTA
1701 AAGAAATCCG ACAAATACCT AATGGAAGAG AGTTTCAAA CTAAGCCCCC
1751 AAAAAAGATG GAAACAACAA AGAGAATGCC TACATATGAA GTCACCACAG
1801 CTCTCACTC CTTCTTGAAG TACCTCAGAG AAACACAGCA GCCAGACATG
1851 CAGCTGCTG TACTCTCCAT TGCTACTGCT GTAGGCTATC AGTTGCTAAA
1901 CAGTATTTT CAGCATCTTC TGGGCTGTGA TGAGTAAAC TCTCTCTTG
1951 ATCAATCGA AATTAACCA CATTAATACC AAGAACTGA AATATTTTC
2001 AATTACAGAG CCCAGGCAAT CTGCTGCTC ACAGCCCTAA GAGCCACAGT
2051 TGAAATCACA GATGTTTCTA CACAAGAGAA AGGACAACOT TTGACATTAA
2101 TACACACACA TATGGGCTCA CTGTGTCTG AAGAAGTTGC ACATGCTCTC
2151 ACAAAACATG GAAACATCA TCACTGCGAA AGGCTGAGAA ATGATTTGAG
2201 ATTATCTATT GAGGGGCACT ATAAAGCCAC CAGCCATTC TTACAAATGG
2251 ATGAGTAAA AAAACAATTG CAAAGTTTNT GCCATGAAA GAAACAGACT
2301 TATTAACAAC AAGGTAATCA AAACAGACA AAAGAAATGA TAGAAATGG
2351 ACAATTTCTG GACTTACTCC AACGTTTACG CCTAGACAAT TACTATCCAA
2401 AAAAAAATA AAAAAAATA AAAAAAATA

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FIGURE 24

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1 ACTAGACTCT GGTAGAGAAA AAGTGCCTC GAGTGGCTG CTGAACAACC
51 TCATGACTCA CCAGAGTGTG GAGCTTTTCA AAAAAGTCA CTATGAAGG
101 TCAGGAACA GCTCATTCCT TATCTAAGT TATTTGTAT GCAAGTGGAA
151 ATAGATGAT ATACAGCTCT TAAAAATGG ATGTTCTTTC AGCTGCTGCC
201 ATCTTGGAA' GCGTCTTTT AAAACAGCTT TTGACAAAA CAGATGTCTG
251 GTTTTCCAA: TGAAGAGAG AATTGAAAG CAGGACTTTC CTGAAACTG
301 AACAGGAAA ACCAGTTCG GCGCTTTC CACATTAA GCTACAGTA
351 CATATCAGT GATCTGCTT CTGAAAGAT CATGAGCAG GATTCTCTG
401 TACCTTCAAG ATGCTGCTG GCAATGTAT AACAGCAGT GCTGCTATG
451 CTAGGCTCT AACAGAGAG TAAATGCGG CCTCAAGAA TCAATAAGA
501 AGAATTGAK GGAACAGCA TAAATGTGT TGAAGCTT GCAAGATG
551 GTGATTAAT CTGCGCTGG ACAGCTTCA ATTTGCTT TGACCTCTT
601 GTGACTTAC CCAATGATA CATCATTTTC CACGCCATA CACTGAACCA
651 GCCATGTA: TGAATCTTC AGCTTACAC TGAAGGAGC ATAGCATTA
701 AATGCTTTC GCTTCTTTCA CAGTATGGG A

SEQ ID. No: 22

FIGURE 24

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LL2-35(ge)

1. GACATTEUAG OCTACACTAC ATTATCAGTG ATCTGCTTC TCCAAGGATC
51. ATTGACAGG ATTCTCTGCT ACCTTCAGAA TGCTGCGCG CAGTGTATTA
101. ACAGCAGTGC CTGCTATTC TACGCTGA ACAACACAT GAACTGCGC
151. CTCAGCAAT CAATAAGAA GACTTEAGG GAAACACAT GAGTGTGCT
201. CCAAGCTTC CCAAGCTGC TACCTACTGC TGCGCTGGA CAGCTTCAA
251. TTCTGCTTT CAGCTCTTG TACTTACAC CAATGATAC ATCATTTTC
301. AACGATAC NCTGACCG CCACTTATT GATCTGTA CATTACCT
351. CCAAGCA TACTTTAA ATGCTTGC CTCTTTGAC ACTAGTGGG

SEQ ID. No: 23

FIGURE 24

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1 TAACTCCAC OCAATTAATG TAAATTAAGT CATTCAATAG OCACTCCAGG
51 CTTTCAATT TATCTTTCCG GTTCTATOT TCTCTGAAT TCTTACCGGA
101 TAACATTTT ACACAGGAA CAGCTATGAC CATGATTAAG CCAAGCTTA
151 ATACATCTA CTATAGGAA AACTTATAG CTTCTAGTA CCGTCTCGGA
201 ATTCTCTCTT CCACTCCAGG GTCTTACGG TCTTAAAGA CACAGAGG
251 GGGCTCCACA GAGGCTCTT TAACTTAAG GAAACAGCA GCGTCTGATA
301 GAGCTATCTT CAGAGCTTG CAGAAAGGG AGATCAACA CTTTCAATAC
351 CTCATCTAGT TCAACACT GTCTCGAGG ACTTACAAT ACTACATCA
401 GTATCTCTT TTTCTCTG TCTCTCTTA CTACCTCTA CAGATTTGA
451 ACTTACGAA TCCAGACT TTCTCGATC TTTCTAGCC AATCGGCT
501 CAGTCAAGT AAGGAAATG GAACTTACC CAGAGTTTA AGATTTGA
551 AAGATCTGA CAGACATA CCGTCTAGT CCACTACTAC ACCACTATT
601 CCTCAGCA CATTCTCT TCTACTTG TCGAATGC ACCATTCAG
651 CAGCTCTT CTTCTTACA GCGCGAGG TTTGATGCG CTGATAGAT
701 OTTCTACAGT GTAAAGACA CCGTCTAGT TCGTCTCAA CAGACATGA
751 GCGATCTCA CAGCTTACA CCGAATTT TCTACTGCC CAGTTTCTA
801 ACCAATCTA ATCACTGA GTTCTCTG ATCAAGAT GAGGAGACT
851 GCGGATCTT CAGCTCTT CCGCTCTGA CCGGATCTT AGGAAATCA
901 TCACTTCTA CAGACAGCT CTGAAAGT ACTTCTCA CAGCACTCT
951 CACTCTCTA TAACTTAAT TTTTGGTAT AAGCAGAG GCGGCTCT
1001 TCTAGAGCA GTTACACTT TCACTCTA CTTCTAGCT GATAGATAG
1051 ACTCTCTCA CACTCTGAC CCGTCTGTA AGAGCAGAT CCGCTCTT
1101 ATCACTCTA TCGACAGCT CCGCAAGCA ATCTCTCTA ACCCTCTCT
1151 ATCACTCTA ACCACAGCA AAGCTCTAG CCGTCTGAA GATCTCTCA
1201 CCGCTCTAG CCGCTCTAG CACTCTCTA CTTCTCTCA CAGCTCTCA
1251 CACTCTGAC CTTCTAGT CACTCTCAA GATATCTAC TTTCTCTCT
1301 AGCTCTGAA TCGCTCTAG GCGCTCTAG CCACTCTCT CTTCTCTAG
1351 AGCTCTCTT CCGCTCTAG AAGACAGG TCTCTCTCT CCGCTCTCT
1401 AAGCTCTCT TCACTCTCT CTTCTCTCT TCTCTCTCT CCGCTCTCT
1451 CTCTCTCT CAGCTCTCT TCTCTCTCT TCTCTCTCT CCGCTCTCT
1501 GTCTCTCT GTCTCTCT TCTCTCTCT CAGCTCTCT CTTCTCTCT

• DD1:6-1a pe

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1551 GGGGCAACG CAOTGGTGTG CATCTGGGAG CTGAGCCTGG TCAAGGCTCG
1601 CCGGAGAGGT CTGAAGCTTC GACAGGCTTT GTATGACAC ACTGAGGCGG
1651 TCACATGTCT GACAGCCTCT GTACCTTCA CCTCTCTGT CAGCGGATCC
1701 CAGGATCCTT CTTGTATCTT GTGGAGCTG GACCACCTCT CTCGTCTGGC
1751 CTGCTGCTCT GTCCACCTGG AAGGCATCTC AGCCATTGCT ATCACTGATG
1801 TCTGAGGAG CATCTCTCTC TGTCCCGAG CCCACTTGT CCTGTGGAAT
1851 GTCAATGAG AGCCCTCTGC CAGTATACC ACAGCTGGG GCGAGAGAG
1901 AACCATACCT TCTCTCTCA TACTAGAGG GCGAGCTGG CATGCAAGCC
1951 ACCTTATCTT CAGCGGCTCT AAGGACCTTA TCTTCTGAT TTGTAAGTCA
2001 GAGGAGCTTA AGATGCTCT TCCAGGCGG GCACTGATG AGGAGGCTC
2051 CAGGAGCTTA CTAAGCTCTA GAGGCTCAA GTGGGCGAG AATCTTGGCC
2101 TGAAGGAGT GCTGATCTC AOTCTCTCT TGAAGTCAA GCGAGGAG
2151 GCAATCTCT CTGTACAGC TCTGCTCTC ACTAGGAGC AGAGCAAGCT
2201 CTTGCTTCTG CATGAGAGG GCGATCTTC TCTGCTCTG CTGATGCTA
2251 GAGAGCTAGT AGCTGAGCG ACCGAGCTGA AAGCTGAGG CCTGCGCTC
2301 GCGAGCTAGT GCTACAGCG ACAAGATGAT GTGTAGCTG GCTGCTTAA
2351 CCGAGCTAGT TTTTGGGGG GCTCCACTC ACACAGTCT CAAGGAGTCC
2401 CTGATCTCTT GCACCTCTA CCTAAGCAT GTCTGTAGT TATGGAGCTT
2451 CTGTAACTAG CATCTTCTA GACACTGAT CTGGAACTG AGCTGCTAT
2501 GGAATCTCT AGCTGCTCA CTTCACCAAG GCTTATTGA CTGGGAAAG
2551 AAGAGGCTGT GGTATCTGT TCATGAAAG CAGGCTCTG TCTTATTTT
2601 ATTAAGCT: CATTCTCTA AAAAAAAAA AAAAAAAAA AAAAAAAAA

SEQ ID. No: 24 (cont'd)

FIGURE 24

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Page 1

Hdd2.Seq Length: 499 August 12, 1997 14:21 Type: N Check: 4083 ..

```
1  CCCATTAAGTTC CCACCTGCCA TGAGGACTGC ACCTGCCGCA GCGATTTTAT
51  GTGAAGCTG1 ATTACTGTAT GGAATGGAG GAGTGAGAA GTGTGGGAT
101 CATTAAGCA1 GGCAGACCA AAAGACAGAG GGATCTTGGG CCATGAATCA
151 CAGCAAGCA1 AGCTCTCCAC CAACATCTG CACTGGACTC CTACAGGAC
201 AAGAAAGCA1 CTATCTCTTT CAGCCCTGA GACTTGGGCT TAACATGATT
251 TTGCAATG1 CTACCTTACC TAATAGAGCA GAGATGTAA TATATTTCTT
301 ATTTTAAAG2 TGTGATGGCT CAGCTGCAT GGTGAGAAC TACTCTCAT
351 TTATTTCTTT CCAAGGCAT AAAGAGAT GACCAAGCA CAGTCTGTCA
401 TCACATCTA3 TCAAAAGAGC TAATGTCCA GTACAACTCT TCAAAAGAAA
451 AAGAAAGAA1 AAGAAAGAG CATAAAGAG ATGTCTTCTG CTTGAAAAA
```

SEQ ID. No: 25

FIGURE 24

Hdd2.

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14-A3-T7, Seq Length: 688 July 24, 1997 11:39 Type: N Check: 9884 ..

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1: CCGGCTGCGC CCGGAATTAA GTGAGTCCAT TTTTGCACAC CGAGAAATA
5: TCGGACGATG GATAGAGAGT TTTTCTGAAC CCTTTGCGAA GAGACTTCT
10: CAGTATCTCT GATGCTAGAN GAGAGCTCA TAATCGTAGA GGACATAATG
15: ATGCTGAGA TTCTTTGACT CATACAGATG TCACTCTTT CCAGACAATG
20: GACCAATGCT TGTCTAATAT GAGAACTAT ATCAGAAAT TAGAAAGAA
25: CTCTGCTCA CTTCAGTGG ATCCAAATG ACATTCATTT TTTTCTCT
30: CAGTTATAC TTATTCGAA ATAGGAGATG AACCGCCAA GGTTTTCT
35: GCTTCACTC AACTCTCTG AACTCTCTG AATCTCTG AACTCTCTG
40: AACTCTCTG GATTCTGACA GTGGGACTAG AAAAATGCT TATTCTCT
45: CATATCCATG AACGAGCTC NTGTTCTTA ATAACTCTG AACTCTCTG
50: AAAGACTGCT AGATGAAAA AGTCCACCC AGGAATTTCA TCCCTTATG
55: AATGAAATG GATGCTCTCT GCTTTTCTG TGAAGGAAT GCCCAATTN
60: AAGGTTTTT AAATTTTCAA ACCCGGAGC AACCAATCC TGGGAAACC
65: CCTGGAATG AAAAATTTT TTGGGCTCTT AAAAAN

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1 (3;5) (325.1; 934) - 11:51:11 gene, NPM-MLF!

SEQ ID. No: 26

FIGURE 24

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34-D7.Seq Length: 651 July 24, 1997 11:37 Type: N Check: 6224 ..

```

1  CAAAABACI TGTGTTAGC AAATAAAAC TGGCCAGTGG CACAACGTC
51  GCGGGGAAT: GCTGTAGTA CTGCCGATTT CAAAAATGCC TGGCTGTTGG
101  GAGGNCAAA GAAATGNNC GCACAGACAG NNNAAAAGGC CGAGAGAGNC
151  GNNTGCCCN: GAAACCGAAG AACCACAGA NCCCTCTCCC CTTTCGCCCC
201  CTTTCGNN: GAACTGACH ANCAANGAA NCGAGANGAN ACCCAGCANA
251  NCCNCCANT CTATGANTC CTGACTGGCT CCANGAAN CATCCCGGC
301  NCGCAGAAA AAATCCCTGG GCGGCGAGA ACTCCCCCA GCGGACTAG
351  AACTGCTTCT TGAACAGCN NTCGAGAAC TGTGTTGCTT NCCAGAGCA
401  NCCAGAGAT GAACTGAC ATGCTGCTT TCTCTGAT NCCGCGCTG
451  GCTATGNC ACCTAAAAAC ACTGCGNCG GAAACCGAAA ANCGAANGAN
501  GCGGNCNCA TNGTGANTC NCGCGAGAC ACCGACTTT CAGCANGGG
551  GCGGCGGNC GCGGCGCTT NTCGTTGCA CTGCGGGGG GAAAGNCCN
601  AAATGCGC GCGGCGGCG GCGGCGGGG GCGGCGGCG TTTCTTTCT
651  N

```

TINUR = NGF1-B / cur 77 beta type transcription factor

SEQ ID. No: 27

FIGURE 24

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Page 1

34-P4.Seq Length: 683 July 24, 1997 11:37 Type: N Check: 1900 ..

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1  GCGTCGTGTT GCACTGCAGA AXCAGGCGCG TGATGTAGCC CATCCTTCCC
51  TTTCAGATG CTGAGGCTGT TTCTTCTCTC ACCCAGAGCC AGCGGQATGC
101 CACTCTTCCC TCGGCGTTGA CCTGTTCTC TCGGCTACC TCGCTCCCG
151 TCTCTTCCG TTCTCTGTG ATGGTCATTO CAGTTTGA GAAGAACA
201 TCTTTTATT TCGGCTCTG AAAAGCTAGT GTAGCTCTC TCACTGTTT
251 GCACTCCATC TCTCTCTC CATTACCTG CTCTTACTG ATAATTTC
301 TCGAATTCT AACTTTTCA TACATTTT TTAACTACT ATATTGATG
351 TCTTTTAAA AAGAAAGTG CATTTTATC CAAATGTT ATTTCTATA
401 GCTTTTCTT TGTATACCA TTCTCTAGC TATCTCTTT TATATTGTA
451 GCAAGAACTC CCATGTTATG GAATCCCACT GTATGATTG TAAACAGAC
501 ATATGTNAGI TCGCTTTTC AAGAAAGG GTGTGTTGA AATCATCCG
551 ANTCTGCCA GAACTGTCT CAAAGGAA CAGCTCTC TCTGTTCTT
601 GCTGTGTTG TATCATCCG CAAAGGAGT CTCTCTCTG AATTTTGGT
651 TGTGTTGTT TACCAAAAA TTTTCTCTG TTN

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DAP-kinase

SEQ ID. No: 28

FIGURE 24

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38-AS-T7.Seq Length: 624 July 24, 1997 11:37 Type: N Check: 1627 ..

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1  AATGACCTC CTTGTATTC CACTTTCGTA NTCATTTCGG TTCTGATCTT
51  GTCAACCCA GCTGACCCG TTCTGACCC GCGATGCCCT GCTTACTATA
101 CTTTCTTTC TAAGGAATG CTGTTTTTTT TTGAGGTTT TCAAAACAT
151 TTGAAAGCA TTTACTTTTT TGACCAGGA CCATGATTT TCAAAAAAT
201 CCGGCGTTC GTGGGTTTT GTTTTTTT TTAATTTTG GTTGCGTTC
251 CTTTTTTT TAATGGGTT GCGCCATGA AATGGTCC CCACTCACT
301 CTCTGATTC GAACGACTG TGAATCCCT CTTGTGCGA AACTGAACA
351 ACTGTCCTT TTTCCACT CCGTGTGAG TTCTGATG TATGTGCT
401 AGACCCGCG GGTGTGCGA CACTGCCCT GGAACCCCA CCGTGCCT
451 CATCTGTC TGTGCGCC CACATAAAA CTTCAAGAC TCCCTGAAA
501 GTTCTTAAA AATTTAAT AATTTGCTC AATTTTACT GGAATAAT
551 AACTTACT CAGCAATT TTTCTTCT AATGGCTGA AAGAAATCT
601 TGTGCGCA TTTCTTTG CCG

```

glycine-rich RNA binding protein

SEQ ID. No: 29

FIGURE 24

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Page 1

38-Q2.seq Length: 641 July 24, 1997 11:37 Type: N Check: 656 ..

```
1  ATCCAGACC AAGCAACAAG GTCTTTGAG GTCTATAATG AAAGATCTCC
51  ATTCTGATGA CAATGAGGAG GAATCAGATG AAGTGGAGGA TAACGACAAT
101 GACTCTGAAA TGGAGAGGCC TGTGATAGA GCAAGCAGCC GAAGTCGAG
151 AGTTAGCTTA AATGATGCGA GCGATAGTGA AAGCAGTTCT GCTTCTTCAC
201 CCCCACATCA CGAAGCTCCA CCAACCTTAC TAAAAACCA CAACACCCAG
251 ATTCTTGLAG TGAAGATCC NTAAAGCAA ACGAATCAG ATAAGCAAAT
301 AAAGATGCT GAATGTGACA AGGCATACCT AGATGAAGTG GTNGAGCTTC
351 CCGGAAATTT NTGACCTTG AGGGAAGAC ACATTTGCA GCGATCCTG
401 AACCTATAG AAGAACTGG GACACTTTCA TATCACCAC ACAACATTTG
451 ATTCTGATCT TTCTCCTCTG GACAAACCA CAGTCCGTTA ACTACAGAT
501 TCCTGGAAC ATCTGACCA TCTGAAAGAT ATAACCACTG GATGCTCTA
551 GAACCAATGT GTTGGGAGG NTGAGGCTT NTGAGTTTG GGTGTGAAT
601 TTTGTTCCX TTTTAAAT GAAACCCCG CAAATGATGC N
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LTG9/MLLT3

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FIGURE 24

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1 GCGAGCCGAC GGAGNDOCTC TACCGTGGAT ACGTUTTCG GGAACCTATC
51 CCTCGAATTA OCCGAGTCAG GCAGAGAGGG GCGGCGGNOT CCTTCGCCCC
101 TTGCTAAGAG GCGCTGCATT GCAAGGAGAG CCGAGCGGCA GATTCTGTCA
151 CAGACGAGGG AGAAGGCTGT AGAGACAAA GCGCTCACAT CCGGACAGC
201 TTCTTCAAG ACGCTCTCTC TCTCCAGTCC AGAGCGGACC CCGGAGCCCC
251 TGAGGCATCC CTTCGCTCTT CGGACACCCC TAGTCATTCA TTGCTAACAG
301 GCGATCATEA GCGAGCCTGT AAGTAGCCAG TACAGCTCCT TTCTTTTCTG
351 GAGATGCCC ATCCACAAAC TGATCTTTC NAGCTGGAA GCGCTGGGCC

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FIGURE 24

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1 ACCGCTGCCA GAAGACGACA GAAGGGAAGC TAAGGCGTCG TCCTGCGCTC
51 TGAACGCGCG OTTGGACGAA CCGCTGTGCG CCGTTGGCGG ACCTNAGCGG
101 AAAAGAGATG CCGGTGCGAG TGGTGCAGGC TGTGCAGCGG GTTCATCTTG
151 AHTCTGACGC TTCTGTANTT TGTCTCAACC ATGCTCTGAG CACAGAAAN
201 GAGGAAGTGA TGGGTCTGTG TATAGGCGAN TTGAATGATG ACATAAGGAG
251 TGACTCCNAA TTTACATACA CTGGAACGGA AATGCGACA GTCCNAGAAA
301 AGATCGATAC CATCAGAATT GTTCATATCC ATTCTGTGAT CATCTTGCGG
351 CTTCTGACA AGANAAANKA CCGTGTNNAI ATTTCTCCAN ANCANCTGTC

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FIGURE 24

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1 GATGCCCGTC TCCACAAGAG TTAAAGTTCC TCCTAATTTT COCTTGTTCG
51 AAGAACTTGA AGAAGACAA AAAAGGATAG GTGATGGTAC TGTTAAGCTGG
101 GOCCTTGAAG ATGATGAAGA CATGACACTT ACAAGGTGGA CAGGCATGAT
151 TATGGGCCA CCAAGGACAA ACTATGAAAA CAGAATATAT AOCCTGAAG
201 TAGAATGTGG ATCTAAATAC CCAGAGCTC CTCCATCAAT TAGATTGTA
251 ACAAAAATTA ATATGAATGG GATCAATAAT TCCAOTGGAA TCGTGGATGC
301 ACGGAGCATA CCAGGATTAG CAAAATGGA AAATTCCTAT AOCATTAAAG
351 TCATACTTCA AGAAGTAAGA CTCTTATGAT GTCCAAAGAA AATATGAANC

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FIGURE 24

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1 ACCOCTGCGA GAAGACGACA GAAAGGCGAG CTCTGCGTC CCAGGAGTC
51 CAOTACACCA CCATGCGCA TTCTGAGCOT CTCTGCGCCC CCGOCTGCTG
101 GTTACGCTGC ACCAGCTTCT CCGGACCAA AAAGGGAATT CTCTGTTTG
151 CTGAMATTAT ACTGTGCGTG GTGATCTTGA TTGCTTCAG TGCATCTACA
201 ACATGCGCCT ACTCTCCCT CTCGCTGATT GAGATGATCT GTGCTGCTGT
251 CTTACTTCTC TTCTACACOT GTGACCTOCA CTCGAAGATA TCATTCTCA
301 ACTGCGCTTG GACTGACTTC TTCAGATCCC TCATAACAAC CATCCTGTAC
351 CTGATCACCT CCATTGTTOT CCTTGTAGAA AGAAGAAOCA OCTCCAGAT

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FIGURE 24

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1 TACGGCTCCG AGAAGACGAC AGAAGGGGCT CCGGCTTCTT CCGGACCTCC
51 GCGTGGCCGC GACACCGGTC TTGCACTCC GGGACCCCGT GCGATCCTTA
101 CCGCGGTGCA CGATGCCCCC GAAAAAGGGA GAGATGAAA TTAAACCGCC
151 TCAATTATT GGAAGATTTC GAACCTCACT GAAAATTOOT ATCCTTGGAT
201 TGCCAAATOT TGGAAATCT ACCTTCTTCA ATGTATTAAC CAATACTCAG
251 GCTTCANCAAG AAAACTTCCC ATTCTGCACT ATTGATCCTA ATGAGAGCAG
301 AGTCCCTGTG CCAGATGAGA GGTTCGACTT TCTTTGCCAG TACCATAAAC
351 CANCAAGCAA GATCCTGCTT TCCTAAATOT AATGATATT GCTGGCCTTG

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FIGURE 24

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1 ACGOCTOCDA GAAGACGACN GAAGGCGTCC TTTTTCCTOC GCOCOCCTTC
51 AOTCCCTCTT GAGTTGGGCT OTCTCNANT CCGGCGGANA CCGGOCACCC
101 OCAGCCATGA CAGAACTGA TGTAAATCCG AAGGCTATC CCTCNCANA
151 TNGCCACCTC ACCAAGAAC TCGTGGACCT TTTTCHACAG TCTTCTTCT
201 ACAANCAGCT TCGAAAGGA CCNATGAACC NCCAAAACCC TCAACAGAG
251 CATCTCTGAN TTCAATGTGA TGCNCCANAN CTGAACCTTG GAGATCTCCT
301 OCACCTCCCT CTCTGTGCA AAAAAANAAT GTCCCTACCT NTTTGTNCHN
351 CCAACAAGCT TTANCAAGG CCTNTGGGTT TCCAGCCNTC ATCCCTGTCT

SEQ ID. No: 36

FIGURE 24

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1 GAAAGGCCCC TAGATACCCG GATACTTCCG GGTATCCTCG AACTGCACTC
51 TCCTGTTCTT OCCACATTTG ACTGAAQAAG GACAACGGGG TCAAAGTTTG
101 AGGACTGACA GATTCTANAC CCAAGCTTCC TCHGCCCCCA NAAAGCCACC
151 ANACCTCACA TGGAAACAAA GGGCCCATOC CCANCCGCGG TCCCTCAGA
201 GGANANGAAA TTCCTGTTTC TTGTNRCOT CACTGOCAGC CTGCGCGCTC
251 TGAAGCTGCC TCTCCTGNTA TCTANCTOT TCGACGTTCC TNGATGTGG
301 AAGCGCCOTT CTGACCCGOT TCTCCACATT GACCTGCGGA TGTGGCTGA
351 CCTCATOCTA GTGGCTCCCC TCNATGCHNA CACTCTGNGO AACT

SEQ ID. No: 37

FIGURE 24

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1 ACCGCTGCCA GAAGACGACN GAANOGGOGC AGGATGGCTC GGTGGTACAG
51 TTCAAGATCA ANAGCATAC CCCACTGAGC AAGCTGATGA AGCCTACTGT
101 GAGAGGCCAGG OCTTGTGAT NANOCAGATT CGATTCCGOT TTGATGGACA
151 ACCAATCAAT GAAACAGACA CTCCACCCCA NCTGGAGATG GAGGATGAGG
201 ACACENTTGA TOTATTCCAG CAGCAGACAG GAGGATCANC CTCCCGAAGG
251 AGCGTCCCAC ACCCAACGOT TGTCTGACC TGTGCTATTG AGCAGTGACC
301 ATGCTNCCAC ACCCA

SEQ ID. No: 38

FIGURE 24

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1 ACGGCTGCGA GAAGACGACA GAAGGCGAGA GCATCATGCG OCTAAGCGGT
51 CGACTGOCAT TGCCGCGCGT CAGACTGTGG GGTCCGCGAG CATGTATCTG
101 ANCTGCTTGG GACCAANAGC TGCACTAGGG TGCTGCTCAT GGTGCGCGCC
151 GGCATCANCA CAGCCAGTGG CATCCCGGAC TTCAGATCCC CAGCGGAGGG
201 GCGTCTACAG CAACCTTCAG CAGTATGACA TCCCGTACCC TGAAGCCATC
251 TTGGAACCTG GCTTTTCTTT TCACAACCCC AAGCCCTTTT TCATGTTGGC
301 CAAAGACTGT MCCCTGAGCA CTACAGGCCA ATCTCACTCA CTACTTCTG
351 AAGCTCCTCC ACGACANOGA CCGCTTCTGC GCTCTIVACA CAAAAATCGA

SEQ ID. No: 39

FIGURE 24

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1 GGCATCAAN COTGCTTTTG GTCATGCCCC ACTCCTCAAA GATGGACCCC
91 TCTACCCCTT CAACACTAAG CCAGCCAGTG CTGAAGGGAA AGAGGCTCT
101 GAGTCCTCT COTTTTACC TCCCTCTCA CTGCTTCAC AGGAAAGA
151 CAAGCCGTT GCTGAACCA TCCCATCTG TAGCTTCTT CTTGGTACAA
201 AAGAACAGAA CCGGAGAA GAGCCGAGG AACTCTCTC CTGCGGAG
251 TCCGCAATA NCGTCATCC ATCGTCTTA AGTTCTCCC CAAAGCTNAC
301 AGTGAGAGTG AAGCCTTACN GTGCTTACA TTGANTGTAA AACATCACT

SEQ ID. No: 40

FIGURE 24

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>SC|LLS-96-full.seq|14350
CCCACGCGTCCGATCTCCTCCAGGGCCACCAAGCACCTCTGAAGAGCCATGTTCCAAGCTGCCGGAGCCGCCAGGGCCAC
CCCCTCTCATGAAGCCAAAGGCAGCAGTGGCAGCAGCACGGTACAGCGGTCTAAGTCCTTTAGCTTGCGGGCTCAGGTGA
AGGAGACCTGTGCAGCCTGCCAGAAGACTGTGTACCCGATGGAGCGGCTGGTGGCAGACAAGCTCATTTTCCACAACTCT
TGTTTCTGTTGCAAACACTGCCACACCAAACTCAGCCTGGGCAGTTATGCTGCAATGCACGGTGAATTTTACTGCAGACC
TCACTTTCAGCAGCTGTTTAAGAGTAAAGGCAACTACGATGAAGGGTTTGGTCGTAAACAGCACAAAGGAGCTCTGGGCCC
ACAAGGAGGTGGACTCAGGCACCAAGACGGCCTGAGACCCCTTTAACACCCATTCCCTCCCAGCACATGGCCTCCCGCTG
GGCAGTGGAAAGGAGATTAACCCGGGGGCGCGGGGTGGGAGAGGATGAGGCTCCCTCACACAGGTTTCAGGCATAAGGCT
CTGCTCCAGGATTCCTTACTTTTCCCATGGGAGGTTGGCGTTGGGAACCAGAATTGGAATTTTCACCATACTGTGTCCTT
TAGTCCACCTCATCTCACCCACGGCTCCCTGGGAGGCCCACAAGCCCAGCTTCCATACTTAGGTGCTTTTCTCCAGCAA
GGAGTCAGCATGCCCTCCTCAGGGTCCCAAGCTCCCTCACTGCCACCTGGGCCTTGTGTACCCCTTGTCTCCCATCTA
CCTCTGCCCTTAGCCTGGTAATGAGCCACAGAGACTGGAAGAGGGAGAGTGCCATNTACTGGGCCTCATAGATGCCACC
TCGCTGAGGGGGGAGGGCTGGGGAAGAGGCAAGACAGCCTGCAGCCTTCAGGGTCTGGGGGTCCCTTGCAACCACAAAGCT
AAAGCTCTTGCTAGAGCCTCAGCTGACAGGGTCGGCAGTAGCTATGCTCCTCCATCTGTTGTGCTGTTCTGTTGTGATCA
ACCCTCTTTTAAAAACATTTAAACAGCAAAAAAAAAAAAAA

SEQ ID. No: 41

FIGURE 24

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>SC|LL5-96-full.seq|14350 Frame 1
MFQAAGAAQATPSHEAKGSSGSSTVQRSKSFSLRAQVKETCAAC
QKTVYPMERLVADKLI FHNSCFCKHCHTKLSLGSYAAMHGEFYCRPHFQQLFKSKGNYD
EGFGRKQHKELWAHKEVDSGTKTA*DPFNTHSLPAHGLPLGSGKEINPGARGGRG*

SEQ ID. No: 42

FIGURE 24

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>SCISA7-full
AGCTCTAGTC CCCAGAGATG TCGCCACTAC TGCTGCTGCT GCTGTGCCTG
CTGCTGGGGA ATTTGGAGCC TGAGGAGGCC AAAGTGATCC GTGTCCCTCT
TCAACGAATC CACCTTGGAC ACAGAATCTT AAACCCACTG AATGGATGGG
AACAGCTGGC AGAGCTTTCT AGGACCTCCA CCTCTGGTGG CAACCCCTCC
TTTGTGCCTC TCTCCAAGTT CATGAACACC CAGTATTTTG GAACTATTGG
TTTGGGAACG CCTCCTCAGA ATTTCAACCGT TGTCTTTGAC ACGGGTTCTT
CCAACTTGTG GGTTCCTGCC ACGAGATGTC ATTTCTTCAG TTTGGCATGC
TGGTTTCACC ATCGCTTTAA TCCCAAGGCC TCCAGCTCCT TCAGGCCCAA
TGGGACCAAG TTTGCCATTC AGTATGGGAC CGGGCGGCTG AGCGGAATCC
TGAGCCAGGA CAATCTGACT ATCGGGGGGA TCCACGATGC TTTTGTGACA
TTTGGAGAGG CTCTGTGGGA GCCCAGCCTG ATCTTTGCTT TAGCCCACTT
TGATGGGATC CTGGGCCTCG GCTTCCCCAC TCTGGCTGTG GCGGGAGTTC
AGCCTCCGCT GGATGCGATG GTGGAGCAAG GGCTGCTGGA GAAACCCGTC
TTCTCCTTTT ACCTCAACAG GGATTCTGAA GGGTCTGATG GGGGAGAGCT
GGTCCTAGGG GGCTCAGACC CCGCTCACTA CGTACCTCCC CTCACCTTCA
TACCAGTCAC CATCCCTGCC TACTGGCAGG TCCACATGGA GAGTGTGAAG
GTCGGCACAG GGCTGAGCCT CTGTGCCCAG GGCTGCAGTG CCATCCTAGA
CACAGGCACA TCCCTCATCA CAGGACCTAG TGAGGAGATC CGGGCCTTGA
ATAAAGCCAT TGGGGGATAT CCCTTCCTGA ATGGGCAGTA CTTCAATCAG
TGTTCCAAGA CGCCAACGCT TCCCCCTGTC TCCTTCCACC TTGGTGGAGT
CTGGTTTAAC CTCACAGGCC AGGACTATGT CATCAAGATT CTTCAGAGCG
ATGTTGGCCT CTGCCTGTTG GGCTTCCAAG CCTTGGATAT CCCCAGCCT
GCGGGACCCC TCTGGATCCT TGGGGACGTC TTTTGGGGC CCTATGTGGC
TGTCTTTGAC CGTGGGGACA AAAACGTCGG CCCGCGCGTG GGAAGTGGCGC
GTGCTCAGTC TCGTTCAACA GACCGGGCAG AAAGAAGGAC TACGCAGGCG
CAGTTCTTCA AAAGACGCCC TGGTTAGGGT ACAAGCTCAC CGGGCCACAG
CAGCTATGCT TCTTTCCAAT TAAACAACT AAAAAAAAAA AAAAAAAAAA
AAAAAAAAA A

SEQ ID. No: 43

FIGURE 24

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>SC|SA7-full Frame 3

MSPLLLLLLC LLLGNLEPEE AKLIRVPLQR IHLGHRILNP LNGWEQLAEL
SRTSTSGGNP SFVPLSKFMN TQYFGTIGLG TPPQNFTVVF DTGSSNLWVP
STRCHFFSLA CWFHHREPNK ASSSERPNGT KFAIQYGTGR LSGILSQDNL
TIGGIHDAFV TFGEALWEPS LIFALAHFDG ILGLGFPTLA VGGVQPPLDA
MVEQGLLEKP VFSFYLNKDS EGSDGGELVL GGSDPAHYVP PLTFIPVTIP
AYWQVHMEV KVGTLGLSLCA QGCSAILDTG TSLITGPSEE IRALNKAIGG
YPFLNGQYFI QCSKTPTLPP VSFHLGGVWF NLTGQDYVIK ILQSDVGLCL
LGFQALDIPK PAGPLWILGD VFLGPYVAVF DRGDKNVGPR VGLARAQSR
TDRAERRTTQ AQFFKRRPG

SEQ ID. No: 44

FIGURE 24

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>SC|DD136|14044

CGGTGACGCAGGACCAGGACTCGCGCGTCCAGCGGAGAAGCAGGAGAAGCCGGCGACCTTGCGCTCTCAGCCTGATCCCT
GTCTTGGCGGCCTGAACATTGCGAGCTGGAGAGATGGCGTTCGTGAAGAGTGGATGGTTACTTCGGCAGAGCACCATTCT
GAAACGCTGGAAGAAGAATTGGTTGACCTGTGGTCAGACGGTCACCTGATCTACTACGATGATCAGACTCGGCAGAGCA
TAGAGGATAAGGTCCACATGCCCCTGGACTGCATCAATATCCGCACGGGGCATGAGTGCCGGGACATCCAGCCTCCAGAT
GGGAAGCCCAGAGACTGTCTGCTGCAGATCGTTTGGCGAGACGGGAAGACCATCAGTCTCTGTGCAGAGAGCACAGACGA
TTGCCTGGCATGGAAGTTTACACTSCAGGATTCCAGAACAACACAGCTTACGTTGGTTCAGCAATCCTGTCTGAAGAGA
CTGCAGTGGCCGCGTCCCCGCTCCCTACGCAACCTATGCTACACCNACCCCTGAGGTCTACGGCTATGGTCCATACAGC
GGCGCATACCCCGCAGGAACCTCAAGTTGTCTATGCCGCCAACGGGCAGGCATATGCAGTGCCATACCAGTACCCGTATGC
AGGAGTTTATGGACAACAGCCTGCCAACCAGTCAATCATCCGCGAGCGGTACCGAGACAATGACAGTGACCTGGCTCTGG
GCATGCTCGCCGGGGCAGCCACCGGCATGGCCCTGGGCTCTCTGTTCTGGGTCTTCTAGAGCCTTCAACATTTTCTGTGC
ATAGCTTCTGTTAGTCTGTGTGCAGTAATTTGATTTGCAGGGCATTCTGTTTGTGACAAGTGTCTTTCATAATAATTT
AAATAGTTCTTTTGAAGGTGGTAATCTAATAATTGTGACTGACCTGCATGGTACCACAAAGAAAGCCCGAGGTATGCTGT
GAGTGAGAGCCTGAGTCTTCCGGGTACTAGCTTGCAACCAAGTCTTCTTAGGGACTTTTGGATGGCTTTATGTAAACAC
ACCCAGTTAAATGGGCAATTTCCGTCCAGTTAGGTGCAGTGTGAATTAAGGGATGGCTTTCCTTGCTATGCCAATACTA
ATACTGCTGATGGAGGAAGATGTGTGCAAGTGTGGTGAGGAGAGTCACAGCTTCTTTAACTGTGGATTCTCTTCTAGACC
CCTGCTGCGTGTACCCTAGGAGCTGTGGGCTGGTGGCTCCTGCAAGACTATGGTGTGAGGACCCTGTAACGTACCTCTT
GGAGCACTTAGGTACCCCTTGAAGCTCCTAGGTATCACCAGCAGGATTGGCTGCTCAGGATGCAGAGGGCCACCCCTCC
CTTTAAAAATTACGCTCCAGTAATCTGCCAGTTTTATTTCTTGTATTCTTCTGTTTGCTTTTCTGGGGATGATTGG
CATTAGTCTGGAGTTAGGAATTGATTGAGTGCCCGGTGGGTGGAGGCATGCAGGGAGCTGTCCAGCGACCTGCTCTCAGT
GTTTGTTTTAGGTATATTGATTGCCAGCTCAGGCTGCAGAGAGCCTATAGAGACTATTTTCTACTTGTAAGAAAGTAT
GGTGAGGGGAATTGAGGAGAGCCTTGTTTGAATGTTCTCCTGCTCAGGCCTCCTGGGGCCCACTGCGTGGTCTGGGGA
GGCTTTTCTCAGCAGTGGAAGGGAGGCCCGTGGCTGTCCAGAGTCTCAGGTTTTGAGTGAGAAATGGGATTGGGTAGA
GCATCTCAGGGATTTGTTCTAATCCCTCATGTTATGGGGATCCAGCCGTGTTCTCAGTCCAGACCCGCTCACCTCAGAAG
AGCTTAAACATTTCTGGTCCCCAAATGTGTGGCACTCTGAGAAGCTCACAATCTGGCTTTCTAACGAAAATTTGTATTT
CTAAATTAGAGAATACATGTTCCACGCATTTAAATTTATGTTCTTTCATGTTTTAAAGCTCCCAAATCCAGCTTTGTG
ACTGGCATATTTTAGTTTTCAAACAGTACCCCGGCACAAAGGTGGGATGGCACAGTGAAGGCCCCCGCCCTCTACTTTGC
ATAGTCTTGTCTTCCAGGGTGCTCCAGGAAGCATTATTCTGACTTTGCTCAGCCCAGTGCATGCGTGCTGCCTTGCC
GCCGTGCTGCTGGGTAGCTCTTCTTGGTCAGATCAAGTCTTCAACAGATCTCCATGTGAGACAGTTGCCAAGTAGATGA
GGTGGTGCCCATAGTGCTTCTCGATACTCCTTGGGGACCTGTTGACACCTGCCCATTTCCAGCTGACATTTGTTTTCT
GTCATCTCTGATAGATGGGATATGTGACAACATGGTACGGACGCCGTTTCAAGTGTGCTTTAATAAGCATGATGCTGATTT
TACATCCTGTGCTGTATGACTGCCATTTGCTCAGAGTGTCAACATTGCTAAAGCTCCGTGCTTTACTTACAAAACACTAA
AACCAGTGGTTAGTGTTCACAGTGATTTTAAATTTTAGAGTTAGTTACTGGCATTCTTAAAGCCATAGAGTACTGAGTCA
CATCCCTGAAGTACTTTTGAACAGAATTGTCTCCTACTGTCCCATGGGTGTGCCCTGCCTGTCTCCTGGCCCCAATGGG
GCTAGCTGTACCAGGCAGCCATAGTTGAGCCTGATCATTCTGTCAACAGTTTGACTTGATTATATACCCAGAATGGAAT
ACATTCTTGGGCATCTCAGTTCCTCAGCCCTGATCCTCATAGACGCCACCCTTTCGATGGCTTTTGGCGCGTCACTTGTA
CCTCAGTGAGTCTGCGATTCTTGAGTTAGAGGGGACGACTTGTCCAGCATTGAGGAACATGTCTCCTCCACTGAGACTT
AAATGATGATGCAGGGCTGGAAGAGGCTGGCTGCTGACACTGCATCGTGGCTGATGTCATTGCTCTCCTAGTTCTTTGAT
TTAAGAACCTTTCATATGGAAGGCTTGAAGCTCCCTCAGATCGTCCCTTGCCAAGAAGGCTGGCTTAGGTCAATTAGTGC
CCACAGTAGCCTTCTGGAGTGTAGCAGTTTCTGCGTTTGGAGACAGAATGGTTCAATTTATTTTCTACATCTGTTGTTG
ACCCCATGCACCCTCTCATTTTGCCTTCCAGTCTACGTAGATGAAAGATGAAAGGCAGAGGATGCAGACAGTCTTCTTTG
TGATTGCTTCTGTTATTCTGTTGCATCTACCGAGCCCGTTTTCTCCCTGTCTGTGCATACAGTATGTTTATAAGTGAAC
TGTTAAATATTAAATGATCACTAACCTAAAAA

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FIGURE 24

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>SC|DD136|14044 Frame 3
MAFVKSGWLLRQSTILKRWKQW
FDLWSDGHLIYYDDQTRQSIEDKVHMPVDCINIRTGHECRDIQPPDGKPRDCLLQIVCRD
GKTISLCAESTDDCLAWKFTLQDSRTNTAYVGSAILSEETAVAASPPPYATYATXTPEVY
GYGPYSGAYPAGTQVVYAANGQAYAVPYQYPYAGVYQQPANQVIIRERYRDNDSDLALG
MLAGAATGMALGSLEWVF*

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FIGURE 24

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>SC|DD116|14045

CACCACAGAGGACCCCTTCAACCTAAGGAAACACCCAGGCTTCGATAGGACCATGCTGCAGAGGTGGCAGAAAAGGGAGA
TCAGCAACTTTGAATACCTCATGTACCTCAACACACTGGCCGGAAGGACCTACAATGACTACATGCAGTATCCCGTGTTT
CCCTGGGTCCCTCGCTGACTACACCTCAGAGATGTTGAACTTGACGAATCCCAAGACTTTCCGGGATCTTTCTAAGCCAAT
GGGGGCTCAGACCAAGGAAAGGAAGTTGAAGTTTACCCAGAGGTTTAAAGATGTTGAAAAGATTGAAGGAGACATGACCG
TGCAGTGCCACTACTACACCCACTATTCTCAGCCATCATTGTGCTTCTACTTGGTCCGAATGCCACCATTACGCAG
GCCTTCTGCTCCTTACAGGGCGGAAGCTTTGATGTGGCTGATAGAATGTTCCACAGTGTAAGAGACACGTGGGAGTCTGC
CTCCAAAGAGAACATGAGCGATGTGAGGGAGCTGACACCTGAATTCTTCTACCTGCCCGAGTTTTTAACCAACTGTAATG
CAGTGGAGTTTGGCTGCATGCAGGATGGAACGACACTGGGGGATGTGCAGCTTCTCCCTGGGCTGACGGGGATCCGAGG
AAATTCATCAGCTTGACAGACAGGCTCTGGAAAGTGACTTCGTGAGCAGCAACCTCCACCACTGGATAGACCTAATTTT
TGGGTATAAGCAGCAGGGGGCCGGCTGCTGTAGAGGCAGTGAACACTTTCCACCCCTACTTCTACGGTGATAGAATAGACC
TGGGCAGCATCACTGACCCGCTGATCAAGAGCACCATCCTGGGCTTCATCAGCAACTTTGGACAGGTGCCAAGCAGATC
TTCATAAACCCCAACCCATCCAGAAACACACAGGGGAAAAACCCAGGGCCTGGAAAGGATGCTTCCACCCCTGTAGGCCT
CCCAGGCCACTCACAGTCTTCTCCACAGCCTGCCAGCACTGAGACCCTCTCAGGTACAGTCAAAGATATGTACCTTT
TCTCTCTAGGGTCGGAATCCCCCAAAGGGGCCATCGGCCACATCGTCCCTACTGAGAAGTCAATCCTGGCAGTGGAGAAG
AACAAGCTGCTGATGCCCCCTCTCTGGAACAGGACCTTCAGCTGGGGCTTTGATGACTTCAGTTGCTGCCTGGGGAGCTA
CGGCTCTGACAAGATCCTGATGACCTTTGAGAACCTGGCTGCCTGGGGTCCCTGTCTGTGCGCTGTATGCCCTTCCCCCA
CGATGATCGTCACATCCGGGGCCAGCGCAGTGGTGTGCATCTGGGAGCTGAGCCTGGTCAAAGGTGCCCCGAGAGGTCTG
AAACTCCGACAGGCCTTGTATGGACACACTCAGGCGGTACATGTCTGACAGCCTCTGTACCTTCAGCCTCCTGGTGAG
CGGATCCCAGGATCGCACTTGTATCCTGTGGGACCTGGACCACCTCTCTCGTGTGGCCTGCCTGCCTGTCCACCGGGAAG
GCATCTCAGCCATTGCCATCAGTGATGTCTCGGGAACCATTTGTCTCCTGTGCCGAGCCCCTTGTCCCTGTGGAATGTC
AATGGACAGCCCCCTGGCCAGTATTACCACAGCCTGGGGCCCAAGGAACCATACGTGCTGCTGCATAGTAGAGGGGCC
AGCGTGGGATGCAAGCCACGTGATCATCAGGGGAGTAAGGACGGAATGGTTTCGGATTTGGAAGACAGAGGACGTGAAGA
TGCCTGTTCCCAGGCAGGCAGTGATGGAGGAGCCCTCCACGGAGCCCCTAAGCCCCAGAGGTACAAAGTGGGCCAAGAAT
CTTGCCCTGAGCCGAGAGCTGGATGTCAGTGTTGCTCTGAGTGGCAAGCCCAGCAAGGCAAGTCTGCTGTGACAGCTCT
GGCCATCACTAGGAACCAAGCAAGCTCTTGTTGGCGATGAGAAGGGCGAATCTTCTGCTGGTCTGCTGATGGGTAGGA
GACAAGGAGCTGGAGGGACCGACCTGAAAGCCTGGAGCCCTGGGGTGGCAGCAACAGGCTACAGGCACAAGATGATGTG
TAGCCTGGGCTGCTTAACCAGAGCAAGTTTTGGGGGGCTCCACTCCACACAGTTCTCAAGGAGTCCCTGATGGTTTGCA
CCGTGTACCCTAAACATGTCTGTAGTCTATGGGACTTCTGTAAGAAGGATCTTGGTAGACACTGATGCTGGAACTGACG
CTGCATGGGAAATTTCTACGTGGCTCACTTCACCAAGGCTTATTGCACTGGGAAAAGAAGAGGGGGTGGGTATTGGTTCA
TGGAACCAACCCCACTGTCTTTATTTTATTAAACTCCATTTTCCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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FIGURE 24

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>SC1DD116114045 Frame 2
TTEDPFNLRKHPGFDRTMLQRWQKREISNFEYLMYLNNTLAGRTYNDYMQYFVFPWVLADY
TSEMLNLTNPKTFRDLSKPMGAQTKERKLKFTQRFKDVEKIEGDMTVQCHYYTHYSSAI I
VASYLVRMPFFTQAFCSLQGGSFVADRMFHSVKSTWESASKENMSDVRELTPEFFYLPE
FLTNCNAVEFGCMQDGTTLGQVQLPPWADGDPRKFISLHRQALESDVSSNLHHWIDLIF
GYKQGGPAAVEAVNTFHPYFYGDRI DLGSITDPLIKSTILGFISNFGQVPKQIFTKPHPS
RNTTGKNPGPGKDASTFVGLPGHSQSFLHSLPALRPSQVTVKDMLFSLGSESPKGAIGH
IVPTEKSILAVEKNKLLMPPLWNRTFSWGFDDFSCCLGSYGSDKILMTFENLAAWGPCLC
AVCPSPMTMIVTSGASAVVCIWELSLVKGRPRGLKLQALYGHQTAVTCLTASVTFSLLVS
GSQDRTCILWDLHLSRVACLPHVREGISAIASDVSGTIVSCAGAHLSLWNVNGQPLAS
ITTAWGPEGTITCCCIVEGPAWDASHVIITGSKDGMVRIWKTEDVKMPVPRQAVMEEPST
EPLSPRGHKWAKNLALSRELDVSVALSGKPSKASPAVTALAITRNQSKLLVGDEKGESSA
GLLMGRRQGAGGTDLKAWS PGVGSNRLQAQDDV*

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FIGURE 24

FIGURE 24

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>SC|DD64|14046 Frame 1
QRQAGQALXALRALQSEGKHQEEAVRRKGAXLRQAMEIPEECWPTAEVSLKDITEIMER
HLSHMERTLSHSQKLSGDGLVRWASGGLVLQGIYKTNHPRS LIQKREELLSVPKQFSLAG
PVHGTEIKTEFSSFQEQAMFTQTIERVGFSSSTPLVKGEGWGLSLEAGMGHNKQTESEDN
YQSHSKQTYFCSARFSYIPLATCHFHINDLELSQAALQELKSIEEILEQTTHHRDGLPLL
RHRAKNFFHRFGSHANQGPVHLGGIYCWKAISEGFKSEHLADVKKQAEESLNIYIMGSYS
GFGVKVGASVNITNSKSKTAFYSKTHLNSQTKVQLSVAKIGGPAEADGIAQWTAGLVASN
QTWSVIDRKLQLVPIWDIILSSHRTEFKNALQLANCLKDHYTALTELAQIQEGEEFLTA
RKEAKLFLKQVKGWEVSDPEEQLRKLVDQMQLSQQIKSYDIWINTCLIDWDLQNFLINI
VNFCNNSPTYKTHFIKSQLCSLLEPHVYKVTNFPQAQSIQWINQSESEELVKITSFSE
FINTLKKTHKYLMEESFKTEPPERVEEAKRMATYEVTTALSSFLKYLRETQQPDMQLLLL
SIATGVGYQLVNSIFQHLLGCDELNFDLQDMENNEHKYQELKNICNYRAQAFVLVTALRA
TVEITDVSTEEKQRLTLIQQHMGSLLEEVAVHLTKHGEHHDWERLENDLRLLEGDYK
ATTHSLQMDEVKKQLQSCHEKKQTYKQGNENRTKEMIENGHFLDLLQRLGLDNYYF

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FIGURE 24

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>SC|19|14042

TCTTCGAAAGCCGGGCTGAGGGGAATCCTGGACAGGGGAATCCTGGACGTGGAGATCGTGAGTCATGGCTGCTTCCCGAG
ACGCTGATGAGATCCACAAGGACGTT CAGA ACTACTATGGGAATGTACTGAAGACATCTGCAGACCTCCAGACTAATGCT
TGTGTCACGCGAGCCAAGCCGGTCCCCAGCTACATCCGGGAAAGTCTGCAGAATGTACACGAAGACGTTAGTTCGAGGTA
TTATGGCTGTGGTCTGACTGTTCCCTGAGCGGCTGGAAAAC TGCCGAATTTTGGATCTGGGTAGTGGGAGTGGCAGGGATT
GCTATGTGCTTAGCCAGCTGGTTGGTGAGAAGGGACATGTCACCGGAATAGACATGACTGAGGTCCAGGTCCAAGTGGCT
AAAACCTATCTTGAACACCACATGGAAAAATTTGGTTTCCAGGCACCCAATGTGACTTTTCTCCACGGCCGCATCGAGAA
GTTGGCAGAGGCTGGGATCCAGAGTGAGAGCTATGGTATTGTCATATCCA ACTGTGTTATCAACCTTGTTCTGATAAAC
AACAAGTCCTCCAGGAGGTCTATCGAGTGCTGAAGCACGGCGGGGAGCTCTATTT CAGTGACGTCTATGCCAGCCTTGAA
GTGCCAGAAGACATCAAGTCGCACAAAGTTTTATGGGGGAATGCCTGGGAGGCGCTCTGTACTGGAAGGATCTTGCCAT
CATTGCCCAAAGATTGGGTTCTGCCCTCCACGTTTGGTCACTGCCGATATCATTACTGTTGAAAACAAGGAGCTCGAAG
GGGTTCTTGGTGACTGTCGCTTTGTGTCTGCCACATTTGCGCTCTTCAA ACTCCCTAAGACAGAGCCAGCCGAAAGATGC
CGAGTTGTTTACAATGGAGGAATCAAGGGACATGAAAAGGA ACTAATTTTCGATGCAAATTT CACATTCAAGGAAGGCGA
AGCTGTTGCAGTGGATGAGGAGACGGCAGCTGTCTGAAGAACTCACGTTTTGCTCCGGATTTTCTCTT CACACCTGTTG
ACGCCTCGCTGCCAGCTCCCCAGGGGCCGTTCTGAGTTAGAGACAAAGGTTCTAATCAGAGATCCATTCAAGCTTG CAGA
GGACTCTGACAAGATGAAGCCCAGACATGCACCTGAAGGCACGGGAGGCTGCTGTGGCAAGAGGAAAAACTGCTAGATCT
ACAGCCAGCGCGGAGCCCACCGGGCTCAAGAGGGTGGCTAAAGGACAGTCACAGAGGCTTCTTAGCCTGCTCTTCGCCAG
TGCACAGATTATGTGAAGGTGGCAAAGCCACCACAAGCTAGACCACTGCTAAGAATAAGAGTGACTTTTAGAGGATGTTA
ATTGAAGGTT CACAGCAAATCGCCTGCTTTTCTATTTCTCTATCTCAGAGTTCTGGTGCCACCTAGTGGTCAGAAGTAGA
ACTTGGAAGCCCAAGGTTTACTCAAAGGGCCAAAGGCATCATCAACGTTGTGAGAATTATCTTCTTCTGGCCTACCACA
GGACACCTCTGGGTTCTTCTCTGTGGTTACCAGGAAGCACAGTACTTACTAAATTTATGCTAACCATGACAAAAGATTGT
CAACTCAAATTTGCTAGGAGTATTCTTTAGGTTGCTGTCTGCAATTTTTTTCTCTGTA ACTGAATGAAAAAGAAAACAAA
TAAAAAATAAATTTGACTTCGAAAAAAAAAAAA

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FIGURE 24

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>SC|19|14042 Frame 2
MAASRDADEIHKDVQNYG NVLITSADLQTNACVTRAKP
VPSYIRESLQNVHEDVSSRYYGGLTVPERLENCRIIDLGS GSGRDCYVLSQLVGEKGHV
TGIDMTEVQVEVAKTYLEHHMEHFGQAPNVTFLHGRIEKLAEAGIQSESYGIVISNCVI
NLVPDKQQVLQEVYRVLKHGGEYFSDVYASLEVPEDIKSHKVLWGECLGGALYWKDLAI
IAQKIGFCPPRLVTADIITVENELEGVLGDCRFVSATFRLFKLPKTEPAERCRRVYNGG
IKGHEKELIFDANFTFKEGEAVVDEETA AVLKNSRFAPDFLFTPVDASLPAPQGPF*

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FIGURE 24

FIGURE 24

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>SC|28LONG|14041 Frame 2
MAVQVLRQMVFYFL
LSLFSLVQGAHSGSPREDFRFCGQRNQTQQSTLHYDQSSEPHIFVWNTTEETLTIRAPFLA
APDIPREFFPEPRGLYHFCLYWSRHTGRLHLRYGKHDYLLSSQASRLLCFQKQEQLKQGA
PLIATSVSSWQIPQNTSLPGAPSFIFSFHNAPHKVSHNASVDMCDLKKELQQLSRYLQHP
QKAAKRPTAAFISQQLOSLESKLTSVSFLGDTLSFEEDRVNATVWKLPTAGLEDLHIHS
QKEEQSEVQAYSLLLPRAVFQQTRGRRRDDAKRLLVDFSSQALFQDKNSSQVLGEKVL
GIVVQNTKVTNLSDEPVLTTFQHQPQPKNVTLCVFWVEDPASSSTGSWSSAGCETVSRDT
QTSCLCNHLYFAVLMVSSTEVEATHKKHYLTLLSYVGCVISALACVFTIAAYLCSRRKSR
DYTIKVMNLLSAVFLLDVSFLLSEPVALTGSEAACRTSAMFLHFSLLACLSWMGLEGIN
LYRLVVEVEFGTYVPGYLLKLSIVGWGFPVFLVTLVALVDVNNYGPIILAVRRTPERVTYP
SMCWIRDSLVSVTNLGLFSLVFLFNLA MLATMVVQILRLRPHSQNWPHVLTLLGLSLVL
GLPWALVFFSFASGTFQLVILYLFISIITSYQGFLIFLWYWSMRFQAQGGPSPLKNNSDSA
KLPISSGSTSSRI*

SEQ ID. No: 54

FIGURE 24

CTCAGGGAAGGCCGATCTTCCGGGTGGAGGGGGAAGCGGCGTGACTGGAGTGGAAATTTTTCCCAACACAACCTTCTCGGA
GGCAACATATTGGAAGGGACTCGGGGAGGCCGGAGTCCAAATGGAAGTGGCTGAAAGAAACTTCTCGCCCTGCTGATTCT
GAGCCCCGCGTCGTGCCGCGCGCCCTCAATTACCTCATCGACCTGTGGTCTTGACAGAACATTCTTCACAATCCAAAAGA
AAAAGCAGACTGGTTCGGGATGTTTGACGGCTATGATAGCTGCAGTGAGGACACAAGTAGGAGCTCCAGCTCTGAGGAGA
GTGAAGAAGAAGTTGCTCCTTTACCTTCCAATCTCCCAATCATCAAGAATAATGGACAAGTCTACACATACCCAGATGGT
AAATCTGGCATGGCTACCTGTGAGATGTGTGGGATGGTGGTGTGCGAGATGCTTTTTACTCTAAAACGAAACGTTTTCTG
CAGTGTTCCTGTTCAAGAAGTTACTCGTCAAACCTCTAAGAAGGCAAGCATTCTGGCCAGACTTCAGGGTAAGCCTCCAA
CAAAGAAAGCAAAAGTCTTCAAACAACCTTTAGTTGCTAAATTAGCTGCCTATGCCAGTATCAAGCTACCTTGCAA
AATCAAGCAAAGACAAAAGCAGGCAATTCTGCAATCTCTGTGGAAGGCTTCAGCTGGGGTAACATCAACAGCAACAG
CTTCATAGCAGCTCCAGTGGCCTGTTTTAAGCATGCACCTATGGGGACCTGCTGGGGTGATATCTCGGAAATGTAAGGA
TAGAAGTTCCCAATACAGACTGCAGTCTACCTACCAAAGTCTTCTGGATTGCTGGAATTATAAAATTAGCAGGTTATAAT
GCCCTTTTGAGATATGAAGGATTTGAAAATGATTCTTCTCTGGACTTCTGGTGCAATATATGTGGGTCTGATATTCATCC
AGTTGGTTGGTGTGCAGCTAGTGGAAAACCTCTCGTTCCTCTAGAAGTGTCAACATAAATATACAACTGGAAAGCTT
TTCTAGTAAAAAGACTTACTGGTGCCAAAACACTTCTCTGATTTCTCACAAAAGGTTTCTGAGAGTATGCAATATCCT
TTCAAACCTTGCAATGAGAGTAGAAGTAGTTGACAAGAGGCATTTATGTGCAACAAGAGTAGCAGTGGTGGAAAGTGTAAT
TGGAGGACGACTACGGCTGGTGTATGAAGAGAGTGAAGATGGAACAGACGACTTCTGGTGCCACATGCACAGCCCCCTAA
TCCACCATATTGGATGGTCAAGAAGCATAGGCCATCGATTCAAGAGATCAGATATTACGAAGAAACAGGACGGACATTT
GATACACCTCCACACTTATTTGCTAAGGTAAAAGAAGTAGACCAGAGTGGAGAATGGTTCAAAGAAGGAATGAAATTGGA
AGCTATAGACCCATTAAATCTTTCTACAATATGTGTTGCCACCATTAGAAAGGTGCTGGCTGATGGATTCTGTGATTG
GGATTGATGGCTCAGAAGCAGCAGATGGATCTGACTGGTCTGTTATCATGCAACCTCTCCTTCCATTTTCCCTGTGGGT
TTCTGTGAAATTAACATGATAGAAGTACTCCACCCAGAGGTTACACAAAACCTTCTTTTAAATGGTTTGACTACCTCAG
GGAAACCGGCTCCATTGCAGCACCAGTAAACCTATTTAATAAGGATGTTCCAAACCACGGATTCCGTGTAGGAATGAAAT
TAGAAGCTGTAGATCTCATGGAGCCACGGTTAATATGTGTAGCCACAGTTACTCGAATTATTCACCGTCTCTTGAGGATA
CATTTTGATGGTTGGGAAGAAGAGTATGACCAGTGGGTAGACTGTGAGTCCCCGACCTCAATCCTGTAGGGTGGTGTCA
GTTAACTGGATATCAACTACAGCCTCCAGCATCACAGTCATCAAGAGAAAGCCAAATCAGCTTCTTCAAACAGAAGAAAA
AGGCTAAGTCTCAGCAATACAAAGGACATAAGAAAATGACCACGTACAGCTGAAGGAGGAGCTGCTGGACGGGGAGGAC
TATAGCTTCTCCATGGAGCATCTGACCAGGAAAGCAATGGCTCTGCCACCGTCTACATCAAACAAGAGCCATGAGGCGA
CTCGGAAACCATGGGCAGGCGGGGCTGTTTACAGGACTGATTTGGAATCAGCCAGCTGTATAGCGGGCTATTCTACTGGG
ACATTTTGCTAAACACAGAAAAAGTTTCAAGTCCAGATTTTTCAGGTGGGGGGGAAACTATTTTGGTGGGGGGGCAATTT
TTCAATTTATAAAGACGGACAATTTTGTGTTGTATTTGAAGCTTTTGAAAGAATTTTGTAAATATTTTCCAAGTTTGGAT
TTATGTGCATTGTTAACAAGAACTGAAATTATACTTTTTTGGTAAGATAAAAGTTTAGGTAGCAGGATTGAAGGAAATG
ATTAAGAAGGATATAGTTGTAAATGCACATGAAGTGTATTACAAATGAACCTTCTTGGTACCTGTTGGGAGATTTTTTG
GATTTTAGAGTTAGGCCAGTCACATCTCCAGCTTCTTTGCTGCGAAAATATGCAACTGAACCCCTCACAGAGGGCTCAT
CACCATGTAGATCACGGAAGGGGTATTAAATTGTGCTCGCTGACGTTTTATTGCAGCCCATTTAACTGTTTGTACAGAAA
CTTTTTTCAATCTGCTAAAATTTATTTGGAGTTGTATATGAAACTAGGAGAACTCTGGATACTTTTATATGCTCTCCTTCA
CGTAAAGATGATTAAAATTTGTCTAACACTAAAGTGTAAACTGGAATGGTTGACAAAGTACACAAGATCTCAGTCTACAT
AAAGGGTTGGGGGAAATTAGTATTTTCTAAGTTTATTCTGTTTTCTTGTAGAAAATCCCATAAAAGGGATATTCTT
GTCAGACCTCTGCCATTTCTTCCATCTGTGAGAGAGCAACGTGATGTAACCACACCTGAGAGCAGGTACTGTCTGTGTT
ACAAAAGAATCACATGGAAAATTTGTTAACCAAAATAAGTGTAGATTTTCAAGATCATTCAGCTCTACTCTTAAGTGTCTT
GAATTTGTTAGAACTGATTTGAAAGAATGTTTCTTAAATTTCCATACACACACACACATATTATGAATTGTCTTTA
ATTGTAGTCAGAATTTATTATAACAATATTTGATTTGGACCATTTTAAACATTCCTTATTTTAAATTCATACGGCTTCC
TTAAGAAGTAGAATGAAGGGTAAATTTGGTGACATGTTTGCTCTCTGCATTTTCTAACCTTCTACCGAATTGTGACTGAC
TCAGAGAGCTCTAGCATTTACCAGTGAGGTTTCAGAACTAACTCTCAGGAATTCATTGCATCTGTTGTAGAAGTGCTTC
TGGGTCTGAGCCTGGCCTCCTCAGAGTGGTAATACTGCCCACTTCTCTGGAAAATAGGCAGGGCTAATGAGAACTAAT
CAAATGATTAACCTCTGCGGCCCTTCAGCCTTTGGAATGCTAAAAA

SEQ ID. No: 55

FIGURE 24

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PCT/US99/19052

SC|sa49p01-full.seq|14351 Frame 2
MFDGYDSCSEDTSSSSSSSEEEVAPLPSNLPI
IKNNGQVYTYPDGKSGMATCEMCGMVGVDAFYSKTRFCSVSCSRSYSSNSKKASILAR
LQGKPPTKKAKVLQKQPLVAKLAAYAQYQATLQNQAKTKAGNSAISVEGFSWGNYSNS
FIAAPVACFKHAPMGTGWDISENVRIEVPNTDCSLPTKVFWIAGIIKLAGYNALLRYEG
FENDSSLDFWCNIGSDIHPVGWCAASGKPLVPPRTVQHXYTNWKAFLVKRLTGAKTLPP
DFSQKVSESMQYPFKPCMRVEVVDKRHLCTRVAVSVIGGRLRLVYEESDGTDDFWC
HMHSPLIHHIGWSRSIGHRFKRS DITKKQDGHFDTPPHLFAKVKEVDQSGEWFKEGMKLE
AIDPLNLSTICVATIRKVLADGFLMIGIDGSEAADGSDWFCYHATSPSIFPVGFCEINMI
ELTPPRGYTKLPFKWFDYLRGTGSIAAPVKLENKDVPHGFRVGMKLEAVDLMEPRLICV
ATVTRIHRLLRIHFDGWEEYDQWDCESPDLNPVGWCQLTGYQLQPPASQSSRESQSA
SSKQKKKAKSQYKGHKKMTTSQLKEELLDGEDYSFLHGASDQESNGSATVYIKQEP*

SEQ ID. No: 56

FIGURE 24

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>SC|sa61|14347
GGGGCTTCACTCTCCAGCCTTGGAGAGCAGTCAGGGAAGGCCTCCAGGAGGAAGCAGCTACCTGTGGCAGGGCAGAGCAG
TGCCTGTACCAGTCTCCCGGAACTTCAGAACTGCGGTGGCCATGGAGTCTGTGGAGCCCCCGGTCAAAGACGGCATCCTC
TACCAGCAGCACGTAAAGTTTGGCAAGAAATGCTGGCGCAAAGTGTGGGCTCTGCTGTATGCGGGAGGCCCATCAGGGGT
AGCTCGGCTAGAAAGCTGGGACGTGCGTGATGGTGGCCTGGGACCAGCAGGCGACAGGTCCACAGGGCCCAGCCGTCGAG
GGGAACGCCGGGTATACGCTTGGCTGACTGTGTATCTGTCCTGCCTGCGGATGGCGAGAGCTGTCCCAGGGACACTGGT
GCCTTCCTGATTACCACCACTGAGCGAAGCCACCTGTTGGCTGCACAGCACCGCCAGTCCTGGGTGGACCCCATCTGTCA
GCTGGCCTTCCCGGGTACCGGAGAATGTTTCGTGAGGATCAGGACAGGCTGAGAATCCAAAAAGGGGCTTTGTTCCCATGG
AAGAAAACCTCTATCTACTCCTCCTGGCAGGAAGTGACCGAATTTCCGGTGATCGTGCAGAAGACAGAAGCCACCTCCCGC
TGCCAGCTGAAAGGACCCTACCTCCTGGTGCTGGGCCAAGATGACATCCAACCTGAGGGAGACATCCAAGCCCCAGGCCTG
TTTTAGCTGGCCCTACCGTTTCCTGCGCAAGTACGGCTCTGACAAGGGTGTGTTCTCGTTTGAGGCTGGCCGCCGCTGTG
ACTCAGGTGAGGGCCTTTTTGCCTTCAGTAGCCCCGCTGCCCCAGACATATGTGGGGTGTGGCTGCCGCCATTGCCCGC
CAGCGGGAGCGTCTTCCAGAGCTGGCCATGTCCCCACCCTGCCCCCTGCCTCGGGCCCTCTCCCTGCCCTCCCTAGAGCC
CCCTGGAGAGCTTCGGGAGGTGGCCCCAGGATTTGAGCTGCCCCACTCCCAGAAAGCTGCCTCTAACTGATCCCGGGCCTC
AAAGCCTACCATTGCTGCTCAGCCCCAACACAAGAAGGACCGGCATCCGGTCTCTATGCGTCCGTGTGCAAGCAGACCAG
CAAGCACACAGGCACGGCGGAGCATTTCTATGAGAACGTGTGCATGCTGGAGGCCAGCCTTGGGCTGACCAATGGGGTCT
CTGAAGCCCAAGAGGGCCCCCTTGGTGGCCGCAGCCCCCTTGGGCAGCCTTATCTACCATAACACTGAGGATCTGAGTTG
GCCGGGCTCGGCCCAGGACAGCAATCTGGAAGCCCAAGTACCGGAGGCTGCTGGAAGTGGAGCTGGATGAGGCCGGAAGCG
CCGGCCGCTCTGGAGCGCAGGCAGGCATCAAGGCCAAGCTGGTGACCCTGCTGACCCGTGAACGGAAGAAGGGCCCCGCC
CCCTGTGACCGGCCCTGAAGGCCTGAGCGGCCAGCCACTGCAGGACAGAGGTGATCACCCAAGACCAGGAACAACCTTCGA
ACATAACCCGTCTACTCTGACCTGCAGGGACAAGCCAGGTGGCCCCGGGAGGAGCCACACTCTGCCCTACCTCCTCCCTC
AGACTGTACAGATTGAACAGTAATAAAGCTTGCCTATCAACTTCAAAAAA

SEQ ID. No: 57

FIGURE 24

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SC|sa61|14347 Frame 2
MESVEPPVKDGILYQQHVKF
GKKCWRKVWALLYAGGPSGVARLLSWDV RDGGLGPAGDRSTGPSRRGERRVIRLADCVSV
LPADGESCP RDTGAFLITTTERS H LAAQHRQSWVDPICQLAFFGTGECSSGSGQAENPK
RGFVPM EENSIYSSWQEVTEFPVI QKTEATSRCLKGPYLLVLGQDDIQLRETSKPOAC
FSWPYRFLRKYGSDKGVFSFEAGRCD SGEGLFAFSSPRAPDICGVVAAAIARQRERLPE
LAMSPPCPLPRALSLPSLEPPGELEVAPGFELPTPRKLPLTDPGPQSLPLLLSPNTRRT
GIRSLCVRVQADQQAHRHGG AFL*RVHAGGQFWADQWGS*

SEQ ID. No: 58

FIGURE 24

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>SC|smc34|14348
GGCCTCCGACATATTGCCCCGAGGAGCTGCGGCGGTGAGCGGAGAGCGCCGGGGGAAGGAGATGGGAGGACGAAGAGGTC
CCAACAGGACATCTTACTATCGAAACCCGCTCTGTGAGCCAGGATCATCAGGGGCTCTGGTGGAGGCCACTCTTCTAGT
GCATCCGTGAGCAGCGTCCGTTCTCGAAGCAGGACCACTTCTGGGACGGGCTCTCCAGCCCTCCGCTGGCCGCGCAGAC
CGTTGTGCCTCTACAGCACTGCAAGATCCCCGAGCTGCCCGTCCAGGCCAGCATTCTGTTTCGAGCTACAGCTCTTCTTCT
GTCAGCTCATAGCCCTGTTTCGTGCACTACATCAACATCTATAAGACAGTCTGGTGGTATCCACCCTCGCACCCACCCTCC
CACACTTCCCTGAACTTCCACCTGATCGACTTCAACTTGCTGATGGTGACCGCCATTGTTCTGGGCGCGCGATTTCATCGG
GTCCATCGTGAAGGAGGCTTCTCAGAGGGGGAAAGGTCTCCCTCTTCCGCTCCATCCTGCTGTTCCCTCACCCGCTTCACG
TTCTCACGGCGACAGGCTGGAGTCTGTGCCGGTCCCTCATCCACCTCTTCAGGACCTACTCCTTCTGAACCTCCTGTTT
CTCTGCTATCCGTTTGGGATGTACATTCCGTTCTGCACTGAACTATGATCTTCGCAAGACGAACCTCTTCACCCACAT
GGCTTCCATGGGACCCCGAGAGGCAGTCAGTGGCCTGGCAAGGAGTCGGGACTACTTTCTGACACTGCGGGAGACTTGGA
AGCAGCATAACGACAGCTATATGGCCCCGGAAGCCATGCCACCCATGCCTGCTGCTTGTACCCAGCCTCATTCGCAAT
GAAGTTGAGTTCCTCAAAATGGACTTCAATTGGCGAATGAAGGAAGTACTTGTGAGCTCCATGCTGAGTGCCTACTATGT
GGCCTTTGTACCTGTGTGGTTTGTGAAGAATACACATTACTATGACAAGCGCTGGTCTGTGAGCTCTTCTGCTGGTGT
CCATCAGTACCTCCGTGATCCTCATGCAGCACTTGTGCTGCCAGCTACTGTGACCTGCTGCACAAGGCCGCGCCGCCCAC
CTGGCGTGCTGGCAGAAGGTGGACCCAGCTCTGTGCTCCAATGTGCTGCAGCCACCGTGGACTGAAGAGTGCATGTGGCC
ACAGGGTGCTGCTGGTGAACACAGCAAGAATGTCTACAAAGCAGTGGGCCACTACAACGTGGCATCCCTCCGATGTCTC
CCACTTCCGCTTCCATTCTTTTTTTCAGCAACCCCTGCGGATCCTCAACATCCTTTTGTGCTGCTGGAGGGTGCCGTCATTG
TCTACCAGCTGTACTCCTTAATGTCCTCAGAGAAGTGGCACCAGACCATCTCCCTGGCCCTCATCCTCTTCAGCAACTAC
TATGCCTTCTTCAAGCTGCTCCGGGACCGCTGGTATTGGGCAAAGCTACTCCTACTCAGCCAGCCCCCAGAGGACCTGG
ACCACCGGTTCTCCTGAGTCCCAGGGTGATCCTCCAAACAACATCCGGGCTTCACCCAGGGGCTTCTTGCCAAAGGGCTC
TTGGGGTTGGGGGGGAGGGATACAAAAACAACAAAAACAACAAAAAACCAACCCACCAGAGCTTTGTATTTT
TGTTACGTACTGTTTCTTTCTTTGATAATTGATGTGGTAAGAAAAAGTCTTATTTTATACTCCAAAAA

SEQ ID. No: 59

FIGURE 24

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SC|smc34|14348 Frame 2
MGGRRGPNRTSYRNP LCEPGSSGASGGGHSSSASVSSVR
SRSRTTSGTGLSSPPLAAQT VVPLQHCKIPELPVQASILFELQLFFCQLIALFVHYINIIY
KTVWWYPPSHPPSHTSLNFHLIDENLLMVTAILGRRFIGSIVKEASQRGKGLPLPLHPA
VPHPLHVL TATGWSLCRSLIHLFRTYSFLNLLFLCYPFGMYIPFLQLNYDLRKTNLFTHM
ASMGP REAVSGLARSRDYFLT LRETWKQHTRQLYGPEAMP THACCLSPSLIRNEVEFLKM
DFNWRMKEVLVSSMLSAYYVAFVPVWFVNTHYYDKRWSCELFLLVSISTSVILMQHLLP
ASYCDLLHKA AAHLACWQKVD PALCSNVLQPPWTEECMWPQGVLVKHSKNVYKAVGHYNV
ASPPMSPTSASISFSATPCGSSTS FCCWRVPSLSTSTP*

SEQ ID. No: 60

FIGURE 24

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PCT/US99/19052

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>SC:AA4.1|15737
GTGAAAGCAGCAGTGGCGCTCTGCTCCCTTCAGAGCACAGCCTGGTGTCAAGGTCCAGGTTCCACCGGCTGCTGCTGTCA
CCGCAGGGGAGTCTAGCCCCCTCCAGAAGGAGACACAGAAGAATGGCCATCTCAACTGGTTTGTTCCTGCTGCTGGGGCT
CCTTGGCCAGCCCTGGGCAGGGGCTGCTGCTGATTACAGGCTGTGGTGTGCGAGGGGACTGCCTGCTATACAGCCATT
GGGGCAAGCTGAGTGCCGCTGAAGCCAGCATCGCTGCAATGAGAATGGAGGCAATCTTGCCACCGTGAAGAGTGAGGAG
GAGGCCCGGCATGTTTCAGCAAGCCCTGACTCAGCTCCTGAAGACCAAGGCACCCTTGGAAGCAAAGATGGGCAAATTCTG
GATCGGGCTCCAGCGAGAGAAGGGCAACTGTACGTACCATGATTGCCAATGAGGGGCTTCAGCTGGGTGGGTGGTGGAG
AGGACACAGCTTATTCAAACCTGGTACAAAGCCAGCAAGAGCTCCTGTATCTTTAAACGCTGTGTGTCCCTCATACTGGAC
CTGTCTTGACACCTCACCCAGCCATCTGCCCAAGTGGCATGAGAGTCCCTGTGGGACCCCCGAAGCTCCAGGTAACAG
CATTGAAGGTTTCTGTGCAAGTTCAACTTCAAAGGCATGTGTAGGCCACTGGCGCTGGGTGGTCCAGGGCGGGTGACCT
ATACCAACCCCTTTCCAGGCCACTACCTCCTCTCTGGAGGCTGTGCCTTTTGCCTCTGTAGCCAATGTAGCTTGTGGGGAT
GAAGCTAAGAGTGAAACCCACTATTTCTATGCAATGAAAAGACTCCAGGAATATTTCACTGGGGCAGCTCAGGCCCACT
CTGTGTGAGCCCCAAGTTTGGTTGAGTTTCAACAACGGGGGCTGCCAGCAGGATTGCTTCGAAGGTGGCGATGGCTCCT
TCCGCTGCGGCTGCCGGCCTGGATTTCGACTGCTGGATGATCTAGTAACCTGTGCCTCCAGGAACCCCTGCAGCTCAAAC
CCATGCACAGGAGGTGGCATGTGCCATTCTGTACCACTCAGTGAACACTACACTTGCCGTTGTCCAGCGGCTACCAGCT
GGACTCTAGCCAAGTGCACTGTGTGGATATAGATGAGTGCCAGGACTCCCCCTGTGCCAGGATTGTGTCAACACTCTAG
GGAGCTTCCACTGTGAATGTTGGGTTGGTTACCAACCCAGTGGCCCCAAGGAAGAGGCCTGTGAAGATGTGGATGAGTGT
GCAGCTGCCAACTCGCCCTGTGCCCAAGGCTGCATCAACACTGATGGCTCTTTCTACTGCTCCTGTAAAGAGGGCTATAT
TGTGTCTGGGGAAGACAGTACCCAGTGTGAGGATATAGATGAGTGTTCGGACGCAAGGGGCAATCCATGTGATTCCCTGT
GCTTCAACACAGATGGTTCCCTTCAGGTGTGGCTGCCCGCCAGGCTGGGAGCTGGCTCCCAATGGGGTCTTTTGTAGCAGG
GGCACTGTGTTTTCTGAACCTACCAGCCAGGCCTCCCCAAAAGGAAGACAACGATGACAGAAAGGAGAGTACTATGCCTCC
TACTGAAATGCCAGTTCTCCTAGTGGCTCTAAGGATGTCTCCAACAGAGCACAGACAACAGGTCTCTTCGTCCAATCAG
ATATTCCTACTGCCTCTGTTCCACTAGAAATAGAAATCCCTAGTGAAGTATCTGATGTCTGGTTCGAGTTGGGCACATAC
CTCCCCACGACCTCCGGCCACAGCAAGCCGACACATGAAGATTCTGTGTCTGCACACAGTGACACCGATGGGCAGAACCT
GCTTCTGTTTTACATCCTGGGGACGGTGGTGGCCATCTCACTCTTGCTGGTGGCTGGCCCTAGGGATTCTCATTTATCATA
AACGGAGAGCCAAGAAGGAGGAGATAAAAGAGAAGAAGCCTCAGAATGCAGCCGACAGCTATTCCTGGGTTCAGAGCGA
GCAGAGAGCCAAGCCCCGGAGAATCAGTACAGCCCAACACCAGGGACAGACTGCTGAAGACTATGTGGCCTTAGAGACAG
CTGCCACTACCTTCAGAGCTACCTTCTTAGATGAGGGGGAAGCCACATCATTCTGAATGACTTGACTGGACTCTCAGCAA
AAAAATTGTGCACCTTCCACTTAAGAACCCTGGTGGCTTGGGATAGGCAGGTATTTCTTGGTGCCTTTGATATGTCTGGG
GGTGAAAGCTGTGTGTTGGTTTGTCAATTGTGGGAGTTTGTGGATATTGACAGACCTCACTCAAACACCCTTTTCAAAT
CCAATAGCAACTGGTTCCTCTGGTTCCTAATTAGGGGGAAGGAGTCAGAGGGGTGGGACAGGGTGGGGGGATGGGGCTT
CAAAGTTTTTTCTTATCACTTGATTATCATCGAAGGAGTTACTGGTGCTAATTACAATGGAAACAGTTCCTTTCCATCA
CAGGACAGACACACCTCAATCCTCCATGGGGTCAACAACCTATATACCCCCAGTGACCCCTTAGGCAAGGACTTGTTGAGA
ACTGCATCACATTTTGACCTGTTCTCAACAGTACCCATCTATTTTCAGGTGGGATCTCTGGACCTTTCCTCCTTCCCATCT
TGTCTGCAATGTGGCAAATGGCTTCTTTTTGCATTTTACTCCGCCCCCACCCTGAAGTTCATTTGCAGATCAGC
GATTAAGTCTGAATTGTGTGGTGGTCAGTCTTGTTCCTTTTGTGAGGGGTATTGTAAATGTTAGTAATTTTCGCCTCAA
GCCCTCAGTAAGAACATAAATATTTTAAATATGTGCGTTTGAAATCTGTTTCATGCATCCTGGAACTGTGGGATGCTCA
GGCAAGAGTGACTTTTAGTCTTTAGTGAATGTTGCCAGAATGTGGGTAGGGAAGGCTCACAGGTACTCTCCTCCTTAG
AGCTACAACATAACATTCTGAGGGGAGTCACAGGGTGCCTTTAAAAGTGGGAGCTATGTCATGCTTTGAGCTTTCTGT
TAAGCACCTCTCCTAATAAACTCTGAAAAA

SEQ ID. No: 61

FIGURE 24

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PCT/US99/19052

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SC|AA4.1|15737 Frame 3
MAISTGLFLLLGLLGQPWAG
AAADSQAVVCEGTACYTAHWGKLSAAEAQHRCNENGGNLATVKSEEEARHVQQALTQLLK
TKAPLEAKMGKFWIGLOREKGNCTYHDLPMRGFSWVGGGEDTAYSNWYKASKSSCIFKRC
VSLILDLSLTPHPSHLPKWHESPCGTPEAPGNSIEGFLCKFNFKGMCRLALGGPGRVTY
TTPFQATTSSLEAVPFASVANVACGDEAKSETHYFLCNEKTPGIFHWGSSGPLCVSPKFG
CSFNNGGCQQDCFEAGDGSFRCGCRPGFRLLDDLVTCASRNPCCSNPCTGGGMCHSVPLS

ENYTCRCPSGYQLDSSQVHCVDIDECQDSPCAQDCVNTLGSFHCECWVGYQPSGPKEEAC
EDVDECAAANSPCAQGCINTDGSFYCSCKEGYIVSGEDSTQCEDIDECSDARGNPCDSL
FNTDGSFRCGCPPGWELAPNGVFCRGTVFSELPARPPQKEDNDDRKESTMPPTMPSSP
SGSKDVSNRAQTTGLFVQSDIPTASVPLEIEIPSEVSDVWFELGTYLPTTSGHSPKPTHE
SVSAHSDTDGQNLFFYILGTVVAISLLLVLALGILYHKRRAKKEEIKEKKPQNAADSY
SWVPERAESQAPENQYSPTPGTDC*

SEQ ID. No: 62

FIGURE 24

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PCT/US99/19052

94/99

SC|HDD2|16960

CCCATTACTTCCACCTCCCATGAGGACTGCACCTGCGGCAGCGATTTTATGTGAACTTGGATTACTGTATGGAAATGGAG -
GAGTGAGAAAGTGTGGGAATCATAAAGAAGGCAGAACCAAAAGACAGAGGGATCTTGGGCCATGAGTCACAGCAAGGAA
AGCCTCCCACCAAACATCTGCACTGGACTCCTACAGGAACAAGAAATGAACTATCGTGTTGAGCCCCTGAGACTTGGGCT
TAACATGATTTTCACATTGTCTACCCCTACCTAATAGAGCAGAGATGTAAATATTATTCTTATTTTAGAGGTGTGATGCCT
CAGCTGCAATGGGTGAGAACTACTCCTCATTATTCTCTTCCAAGGCAATAAAAGAGAATGGACCAAAGACAGTCTGTCA
TCACATCTAGTCAAAAGAGCTAATGTCGCAGTACAACCTCTTCAAAAGAAAAAGAAAAACAAGAAAAAAGTAATAAACAG
ATGTGTTCTGCTTGAAAAA

SEQ ID. No: 63

FIGURE 24

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PCT/US99/19052

95/99

>SCIHDD2116960 Frame 2
MRTAPAAAILCELGLLYGNGGVRKCGNHKRRQNQKTEGSWAMSHSKESLPPNIC
TGLLQEQEMNYRVQPLRLGLNMIFTLSTLPNRAEM*

SEQ ID. No: 64

FIGURE 24

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ACGGAGACAGCTGCAGCGGGGACAGTATTCAGGACAGCTACACCGGCATGGAAAACCTCCGACAAGGACGCCATGAACAGC
CAATTTGCTAATGAAGATGCCGAAAGTCAGAAGTTCCTGACAAATGGGTTTTAGGGAAGAAGAAGCTAGCCGATTACGC
GGATGAGCATCACCTTGAATGACTTCCTTTGGAATGTCTCATTTAACCTGAGCAACGCCATCATGGGCAGTGGGATCT
TAGGCTTGTCTATGCCATGGCCAACACCGGGATCATCCTTTTATAATCATGCTGCTTACTGTGGCAATACTCTCGCTC
TACTCGGTTACCTTTTGCTGAAGACAGCCAAGGAAGGAGGGTCTCTAATCTATGAAAAATTGGGCGAGAAAGCATTGG
ATGGCCTGGGAAAATTGGAGCCTTCATCTCTATTACAATGCAGAACATTGGAGCCATGTCAAGCTACCTCTTCATCATT
AGTACGAACTGCCTGAAGTAATCAGAGCATTTCATGGGACTTGAAGAAAACACTGGGGAATGGTACCTCAACGGCAACTAC
CTCGTCTTATTTGTGTCCGTGGGGATCATCCTCCCGCTCTCTCTCCTTAAAAATTTAGGCTACCTTGGCTACACCAAGTGG
ATTTTCTCTCTCCTGCATGGTGTTCCTCGTCAGTGTGGTGATTACAAAAATTCCAAATTCCTGCCCTCTGCCTGCTC
TGGATCACAAACCGGAAATCTGACGTTCAACAACACACTTCCGATTCACATGATCTCGCTGCCTAATGACTCGGAGAGC
TCGGGTGTGAACCTTCATGATGGATTACGCTCACCACAACCCAGCTGGGCTGGATGAGAAGCAGGTTCGAGGCCCTCTTCA
CAGCAATGGCGTGGAGTACGAAGCCAGGGTGTGAGAAATGCCAACCAAAATACTTTGTGTTCAATTCGCGGACGGCCT
ATGCAATCCCAATCCTGGCTTTTGTCTGTGCCACCCTGAGGTCTTCCCATCTACAGCGAGCTTAAAGATCGATCC
CGCAGAAAGATGCAGACGGTGTCCAACATTTCCATCTCAGGCATGCTCGTCATGTACCTTCTTGGCGCCCTCTTTGGTTA
TCTGAGCTTCTACGGGGACGTTGAAGACGAGCTGCTGCATGCTTACAGCAAGGTCTACACATTTGATACGGCTCTTCTCA
TGGTGGCCTGGCAGTCTGGTGGCAGTGACACTGACCGTGCCCATCGTGCTGTTCCCGATCCGTAATTCGGTGATCACA
CTGCTGTTTCCAAGGAAACCTTCAGCTGGCTGAAGCATTTCGGGATCGCTGCAATCATCATCGCACTCAACAACATCCT
GGTCATCCTCGTGCTTACCATCAAATACATCTTTGGATTTCATAGGGGCTTCTTCTGCCACTATGCTGATTTTCATTCTTC
CGGCTGCGTTTTATCTCAAGCTCGTCAAGAAAGAACCTCTAAGATCACCCAGAAAGATTGGGGCTTTGGTCTTCTTGTG
ACTGGAATTATTTTCATGATGGGAAGCATGGCGCTCATTATACTCGACTGGATCTACAACCCGCCGAATCCCAATCACCA
CTAATCCCGGGGAGACGCGTCTCCACTGGAAACAGCTGAAATTGTCTGAAGGACATTTTAGTTGTCTTGATTGGGATGTT
AGTCTGAGGAATTAGCAAGATTCCAAAGACGTTTTTCTAGCTCTATCAGCACACATTTTAACCCAGGCCGTGCAGTGCAG
TGTGTGATGCCCGAGTTGTGTTTGCAGCAGCTGTGCAAGCTGAAGCCTGTTGGCTGCGTGTGTTGGTCAGCAGACAATAG
CCTGTCCCCCATGGTCACTCCACTTCTCTCCACCCCCAGATTAAACAGGTAATTCTACTCTCAGAACATCAGACAAAGAC
CTCCTGGTTGGGATACTTGTGGAAGAGAAAATTATGGGTTTTGTTGGGAATGGTTTTGTTGGGAATGGTGAAGGATGCAT
TAAAAATTCTGTGCGAAGTATCATCAGTTACGGCCATCTCTCACTCTACACCAACACTAAGGGTGGGTTGACTAGCTGAG
GCAGGGGGATATCTTGGGCTGTCCCTGTGAGGATCATGACGTATGACGGTTGCCAGTATAGAGTACTTCATTTCAATACT
CAAGGAATAGTTTGGCCAACCTGCTTATTACACCGAGTTAGTGAATTTTCATTCTTTTTATTTTTTGGTATGGCAAATGT
TCAAACATCTCACTAAACATAGAGGGGGTTATTTAGTTTATCATGAAACAACCTACCAAAAAGTAATGGTTTTTAACATC
TGCCTTTTCATGTTGTTTCTGATATCTTTGTTCCACTTTGTCTTTGAACAAGGCTTCCCTCTCGTCTTCCGTTCTCACT
CTTCGTAATTTTCAGAAAAACCACAGTCCGTGTGGGAGACACACTACCCAGTATTGTTTGATACATCTCTATTTGATAA
ACATTCAGTGCAGGAACTGTGATTTTGCTATATGTTTGTGTACATAATCTCATTCTGCAGTTATCAGAACGTTGACATA
TGGGACATTGGATTTTTATTTTTTACATATGTAGGTTTTTTTCTTCACAGACAAAATGTTTATATCATCAGGGTGGGGC
AGGGAATTAAGCTGGTGGGCTCAAAAATCCATGGGTACTTATCTGTCCATTGGAGACATCTAAAAATTAAGTCAAAGTT
GTGCATAGTTCAGTAATGCTCTTCACTGTTTACAAGACTATAATCATCTCAGCAAAGTAGCGAAGGAAGGGTTTGTAAAT
AATTTTCAGTGCTAACAATGGTCTTTGAATTTAACATGTCTAGAATAGGGTTTAGTTTATTTAGTTTACATCGGAACCTT
GGTGACATTCATTTGCCATTAATAGAACATCGTGTGGTTAGGAATAAATGCACCAACATGACCCAAAACCACATTGCTCA
CATCCATGGACGCTTACACGGGATGGAGTTTCAGACTCCGCCCCTGCCTAGAATCTCTGGCATTGACTTCTTTTTTTCA
TTACTGGTTGTATATTTTACATTTTCAGTAAAAAGCCAAGTCTGAAATGCAAGTATCCTACAGGTAGTGATATACTGTCAC
ACCAAAGCAGTTTTTCAACAGTGTGCCTAAGAAACATATATTTGTTTACTGTGTCTATTTTCTGGACCTTAAGACTATACA
ATGGTGTGCTTTGTGATAGAAATTGAGCATTACTAGAACTACCATAACTTCGGAGTTATTATCATTTGGGAGGGAAAAA
AATCACCAATAGGATCTTTAAGATGTCTTTCTATTTTCATTTCATCCAAGAAATAAATCAAGATAGAAAGCAGAAAGGGT
GTGCTGCAATATTAAATTATTATTTTATCTGAACATATTAATGTGAATATAAAAGATTAATCCAAAAATATTGGAAACC
TGTGAAATCTGTACGTATTTCCGATCTTTAATCTTAGAGCAGCTAACCTGGAAGTATCCACTTGTGTAGTTAGATATTCA
AGAGCAATATCACAGTGCCAAAGACTTCATTCACACAGCACCGATCATTTGTGTGGCCGTTTTTCAGGATTAGAGTTCACA
CAGACTCCAGGGCAGATACTCAGAATACCGTTTTCTGGTAAATTTAACCCATTTGTAAACAGATACAAATTTTATTTCT
TACATCATTTATAAGACGGTTCAATGTACTGGGGGATTTTTTTTATTACAGACTGTGTATTGGTATATAATAATAAAC
TTTTCAAATGAAAAAA

SEQ ID. No: 65

FIGURE 24

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PCT/US99/19052

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SC|aftpl|15065 Frame 2
MDPMELNNVSI EPDGDSCSGDSIQDSYTG MENS DKDAMNSQFANEDA
ESQKFLTNGFLGKKKLADYADEHHPGAT SFGMSSFNLSNAIMGSGILGLSYAMANTGIIL
FIIMLLTVAILSLYSVHLLLKTAKEGGLIYEKLGKAFGWPGKIGAFISITMQNIGAMS
SYLFIIKYELPEVIRAFMGLEENTGEWYLN GNYLVLFVSVGII LPLSLLKNLGYLGYTSG
FSLSCMVFFVSVVIYKKFQIPCP LPA LDHNNGNLT FNNTLP IHMISLPNDSESSGVNFMM
DYAHNPAGLDEKQVAGPLHSNGVEYEAQGA EKCPKYFVENSRTAYAIPI LAFAFVCHP
EVLPIYSELKDRSRKMQTVSNISISGMLVMYLLAALFGYLSFYGDVEDELLHAYSKVYT
FDTALLMVRLAVLVAVTLTVPIVLP IRTSVITLLEPRKPFSWLKHFGIAAIIIALNNIL
VILVPTIKYIFGFIGASSATMLIFILPAAFY LKLVKKEPLRSPQKIGALVFLVTGII FMM
GSMALIILDWIYNPPNPNHH*

SEQ ID. No: 66

FIGURE 24

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PCT/US99/19052

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>SC|F77|12685

AGCGACATGGCCCCGCCCCGCGCTCCAGGCCCAGCCTCCAGGCGGCTCTCAACTGAGGTTCCCTGCTGTTCCCTGCTGCTGTT
GCTGCTGCTGCTGTCATGGCCATCGCAGGGGGACGCCCTGGCAATGCCTGAACAGCGACCCTCCGGCCCTGAGTCCCAAC
TCAACGCCGACGAGCTACGGGGTCTGCTTCCAGGACCTGCTGAGCCGGCTGCATGCCAACCAGAGCCGAGAGGACTCGAAC
TCAGAACCAAGTCCTGACCCAGCTGTCCGGATACTCAGTCCAGAGGTGAGATTGGGGTCCCACGGCCAGCTGCTACTCCG
CGTCAACCGGGCGTCGCTGAGTCAGGGTCTCCCCGAAGCCTACCGCGTGCACCGAGCGCTGCTCCTGCTGACGCCGACGG
CCCGCCCCCTGGGACATCACTAGGCCCCCTGAAGCGTGCGCTCAGCCTCCGGGGACCCCGTGCTCCCGCATTACGCCTGCGC
CTGACGCCGCCTCCGGACCTGGCTATGCTGCCCTCTGGCGGCACGCAGCTGGAAGTGCCTTACGGGTAGCCGCCGGCAG
GGGGCGCCGAAGCGCGCATGCGCACCCAAGAGACTCGTGCCCACTGGGTCCGGGGCGCTGCTGTCACTTGGAGACTGTGC
AGGCAACTCTTGAAGACTTGGGCTGGAGCGACTGGGTGCTGTCCCCGCGCCAGCTGCAGCTGAGCATGTGCGTGGGCGAG
TGTCACCACTGTATCGCTCCGCGAACACGCATGCGCAGATCAAAGCACGCCTGCATGGCCTGCAGCCTGACAAGGTGCC
TGCCCCGTGCTGTGTCCCCTCCAGCTACACCCCGGTGGTTCTTATGCACAGGACAGACAGTGGTGTGTCACTGCAGACTT
ATGATGACCTGGTGGCCCCGGGGCTGCCACTGCGCTTGAGCACCGGGGCCCTGCTCCTCACCTACACTCCCCTTCAAGGATG
CTATTTATATTTGTATTTATTAATATTATTAATTTATTGGGGTCCGGCTGGGTGGATGGATTGTGTATTTATTTAAACT
CTGCTAATAAAGGTGAGCTTGGTTTCAAAA

SEQ ID. No: 67

FIGURE 24

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PCT/US99/19052

99/99

>SC|F77|12685 Frame 1
MAPPALQAQPPGGSQLRFLLELLLLLLLLLSWPSQGDALAMPEQRPSGPESQLNADEL
GRFQDLSRLHANQSREDSNSEPSDPAVRILSPEVRLGSHGQLLLRVNRASLSQGLPEA
YRVHRALLLTPTARPWDITRPLKRALSRLGPRAPALRLRLTPPPDLAMLPSGGTQLELR
LRVAAGRGRSAHAHPRDSCPLGPGRCHLETVOATLEDLGWSDWVLSRQLQLSMCVGE
CPHLYRSANTHAQIKARLHGLQPDKVPAPCCVPSSYTPVVLHRTDSGVSLQTYDDLVAR

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FIGURE 24