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(54) 【発明の名称】 神経芽細胞腫において単離された核酸

(57) 【特許請求の範囲】

【請求項1】

配列表の配列番号229に記載の塩基配列、又は、その一部配列であって少なくとも40個以上の連続した塩基配列、からなる核酸を含有することを特徴とする神経芽細胞腫検出用診断薬。

【請求項2】

n b l a 1 0 6 7 7 m - f (5' -cataatcttctccggttcac-3') 及び n b l a 1 0 6 7 7 m - r (5' -gtctggtatttccgtgaggttt-3') の一対からなるプライマーセットを有効成分とするヒト神経芽細胞腫の予後の診断キット。

【発明の詳細な説明】

技術分野

本発明は、予後良好な、および不良なヒト神経芽細胞腫との比較において、予後良好なヒト神経芽細胞腫で発現が増強していることを特徴とする核酸に関する。

背景技術

(腫瘍形成と遺伝子)

個々の腫瘍にはそれぞれの個性があり、発がんの基本的な原理は同じであっても、その生物学的特性は必ずしも同じではない。近年、がんの分子生物学や分子遺伝学が急速に進歩し、発がんやいわゆる腫瘍細胞のバイオロジーが遺伝子レベルで説明できるようになってきた。

(神経芽細胞腫)

神経芽細胞腫は末梢交感神経系細胞に由来する交感神経節細胞と副腎髄質細胞から発生する小児癌である。この交感神経系細胞は発生初期の神経堤細胞が腹側へ遊走し、いわゆる交感神経節が形成される場所で分化成熟したものである。その一部の細胞はさらに副腎部へ遊走し、先に形成されつつある副腎皮質を貫通して髄質部に達し、そこで髄質を形成する。神経堤細胞はほかの末梢神経細胞の起源ともなっており、後根神経節（知覚神経）、皮膚の色素細胞、甲状腺C細胞、肺細胞の一部、腸管神経節細胞などへ分化する。

（神経芽細胞腫の予後）

神経芽細胞腫は多彩な臨床像を示すことが特徴である（中川原：神経芽腫の発生とその分子機構 小児内科 30, 143 1998）。例えば、1歳未満で発症する神経芽細胞腫は非常に予後が良く、大部分が分化や細胞死を起こして自然退縮する。現在、広く実施されている生後6か月時の尿のマススクリーニングで陽性となる神経芽細胞腫の多くは、この自然退縮を起こしやすいものに属する。一方、1歳以上で発症する神経芽細胞腫は悪性度が高く、多くの場合、治療に抵抗して患児を死に至らしめる。1歳以上の悪性度の高い神経芽細胞腫は体細胞突然変異（Somatic mutation）が起こり、モノクローナルであるのに対し、自然退縮する神経芽細胞腫では生殖細胞突然変異（germline mutation）のみの遺伝子変異でとどまっているとの仮説もある（Kudson AG等：Regression of neuroblastoma I V-S：A genetic hypothesis. N Engl J Med 302, 1254（1980））。

（神経芽細胞腫の予後を推定する遺伝子）

最近の分子生物学的研究の進展により、神経成長因子（nerve growth factor：NGF）の高親和性レセプターであるTrkAの発現が分化と細胞死の制御に深くかかわっていることが明らかとなってきた（Nakagawara A. The NGF story and neuroblastoma. Med Pediatr Oncol 31, 113（1998））。Trkは神経栄養因子の高親和性受容体で、膜貫通型受容体であり、Trk-A、B、Cの3つが主なものである。

Trkファミリー受容体は、中枢神経および末梢神経系において、特異的な神経細胞の分化と生存維持に重要な役割を果たしている（中川原等：神経芽細胞腫におけるニューロトロフィン受容体の発現と予後 小児外科 29：425-432、1997）。腫瘍細胞の生存や分化はTrkチロシンキナーゼやRetチロシンキナーゼからのシグナルで制御されている。なかでも、TrkA受容体の役割は最も重要で、予後良好な神経芽細胞腫ではTrkAの発現が著しく高く、これからのシグナルが腫瘍細胞の生存・分化、または細胞死（アポトーシス）を強く制御している。一方、予後不良神経芽細胞腫では、TrkAの発現が著しく抑えられており、これに代わってTrkBあるいはRetからのシグナルが生存の促進という形で腫瘍の進展を助長している。

また、神経の癌遺伝子であるN-mycの増幅が神経芽細胞腫の予後に関連していることが明らかになってきた（中川原：脳・神経腫瘍の多段階発癌 Molecular Medicine 364, 366（1999））。この遺伝子は神経芽細胞腫で初めてクローニングされたが、正常細胞や予後良好な神経芽細胞腫では通常1倍体当たり1つしか存在しないのに対し、予後不良の神経芽細胞腫においては数十倍に増幅されるのが見つかった。

しかしながら、現在までに神経芽細胞腫に発現されている癌遺伝子はN-myc以外に知られておらず、その予後の良不良に関する遺伝子情報に関してもN-mycとTrkA以外についてはほとんど知られていなかった。

発明の開示

本発明は、上記従来技術の有する課題に鑑みてなされたものであり、神経芽細胞腫の予後良不良に係る遺伝子配列を明らかにし、その遺伝子情報の提供および予後良不良に関する診断を可能とすることを目的とする。

本発明者らは鋭意研究した結果、ヒト神経芽細胞腫の予後を検定し、予後良好および予後不良の臨床組織の各々からcDNAライブラリーを作製することに成功した。この2種類

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の cDNA ライブラリーから各々約 2400 クローンをクローニングし、神経芽細胞腫の予後の良悪によって分類した。

さらに本発明者は、分類された遺伝子のうち、いくつかで神経芽細胞腫の予後良好な臨床組織でのみ発現が増強している遺伝子を見いだした。

かかる知見に基づき、本発明者は少なくとも予後良好な臨床組織でのみ発現が増強している遺伝子を検出およびクローニングするための塩基配列情報を提供することを可能とした。

さらに、当該領域の塩基配列情報に基づき、予後同定の方法およびそのために使用可能な腫瘍マーカーを設計することを可能とする塩基配列情報を提供することを可能とし、本発明を完成した。

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すなわち、本発明は、予後良好な、および不良なヒト神経芽細胞腫との比較において、予後良好なヒト神経芽細胞腫で発現が増強していることを特徴とする配列表の配列番号 1 から 366 のうちのいずれか一つに記載の塩基配列からなる核酸を提供することを目的とし、さらに、配列表の配列番号 1 から 366 に記載の塩基配列のうち、いずれかの塩基配列の一部からなる核酸を提供することを目的とする。また、上記核酸と、もしくはその相補的な核酸とストリンジェントな条件下でハイブリダイズすることを特徴とする単離された核酸を提供する。

本発明の核酸は、予後良好および不良な神経芽細胞腫の比較により、予後良好な神経芽細胞腫においてのみ発現の増強が認められたものであり、これらの核酸はヒト神経芽細胞腫の予後の診断に用いることができることを特徴とする。その目的で好適な核酸は、配列表の配列番号 121、125、132、133、165、171、187、188、210、229、232、273 および 281 のいずれかに記載の塩基配列からなる核酸、或いはその関連の核酸（塩基配列の一部からなる等）である。それらのうち特に好適な核酸は、配列表の配列番号 121、125、132、165、171、187、188、229、232、273 および 281 のいずれかに記載の塩基配列からなる核酸、或いはその関連の核酸（塩基配列の一部からなる等）である。

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また、本発明は、配列表の配列番号 1 から 366 に記載の塩基配列の一部または全部からなる核酸のうち少なくとも一つの核酸を含有することを特徴とする神経疾患検出用診断薬を提供する。このような腫瘍検出用診断薬としては、具体的には、例えば、前記核酸を用いて製造した DNA チップやマイクロアレイが挙げられる。そこで、本発明は、配列表の配列番号 1 から 366 に記載の塩基配列の一部または全部からなる核酸を複数個含むことを特徴とするマイクロアレイ用組成物をも提供する。このような組成物は、好ましくは全ての核酸（すなわち、各々が配列表の配列番号 1 から 366 に記載の塩基配列の一部または全部からなる計 366 個の核酸）を含む。より好ましくは、配列表の配列番号 121、125、132、133、165、171、187、188、210、229、232、273 および 281 に記載の塩基配列の一部または全部からなる計 13 個の核酸を含む組成物である。

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さらに、本発明に従えば、上記核酸と、もしくはその相補的な核酸とストリンジェントな条件下でハイブリダイズすることを特徴とする単離された核酸であって、DNA であるものも提供される。このような核酸（DNA）の一对からなるプライマーセットを有効成分とするヒト神経芽細胞腫の予後の診断キットもさらに提供される。

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加えて、本発明は、神経芽細胞腫の臨床組織サンプルから配列表の配列番号 1 から 366 のうちいずれか一つに記載の塩基配列からなる核酸の有無を検出することを特徴とする、ヒト神経芽細胞腫の予後の診断方法を提供する。

発明を実施するための最良の形態

本発明における「核酸」という用語は、例えば DNA、RNA、または誘導された活性な DNA もしくは RNA でありうるポリヌクレオチドを指し、好ましくは、DNA および/または RNA をいう。

「ストリンジェントな条件下でハイブリダイズする」という用語は、2つの核酸断片が、サムブルックら（Sambrook, J.）の「大腸菌におけるクローン遺伝子の発現（

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Expression of cloned genes in E. coli)」(Molecular Cloning: A laboratory manual (1989)) Cold Spring Harbor Laboratory Press, New York, USA, 9. 47-9. 62および11. 45-11. 61に記載されたハイブリダイゼーション条件下で、相互にハイブリダイズすることを意味する。

より具体的には、「ストリンジントな条件」とは約45℃にて6.0×SSCでハイブリダイゼーションを行った後に、50℃にて2.0×SSCで洗浄することを指す。ストリンジエンシーの選択のため、洗浄工程における塩濃度を、例えば低ストリンジエンシーとしての約2.0×SSC、50℃から、高ストリンジエンシーとしての約0.2×SSC、50℃まで選択することができる。さらに、洗浄工程の温度を低ストリンジエンシー条件の室温、約22℃から、高ストリンジエンシー条件の約65℃まで増大させることができる。

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本明細書でいう「単離された」とは、組換えDNA技術により作成された場合は細胞物質、培養培地を実質的に含有せず、化学合成された場合には前駆体化学物質またはその他の化学物質を実質的に含まない、核酸またはポリペプチドを指す。

本明細書でいう「予後良好」とは、ヒト神経芽細胞腫のうち、腫瘍が限局して存在するか、または退縮や良性の交感神経節細胞腫になった状態を指し、N-myc その他腫瘍マーカーから判断して、悪性度が低いと判断される。本発明の好適な実施の形態では、病期1または2、発症年齢が1歳未満、手術後5年以上再発なく生存し、臨床組織中にN-mycの増幅が認められないものを予後良好としたが、このような特定の例には限定されない。また、本明細書で使用する「予後不良」とは、ヒト神経芽細胞腫のうち、腫瘍の進行が認められる状態を指し、N-myc その他腫瘍マーカーから判断して、悪性度が高いと判断されるものである。本発明の好適な実施の形態では、病期4、発症年齢が1歳以上、手術後3年以内に死亡、臨床組織中にN-mycの増幅が認められたものを予後不良としたが、このような特定の例には限定されない。

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本発明の核酸は、ヒト神経芽細胞腫の臨床組織より見出されたものであり、かかる核酸は以下のような特徴を有する。

神経芽細胞腫はヒトでは2種類しか知られていない神経細胞そのものの腫瘍の1つであり、そこで発現している遺伝子を解析することは、神経細胞のバイオロジーを理解する上で非常に大きな知見をもたらすものと考えられる。すなわち、脳や末梢神経から、部位特異的な均質な組織を得ることは極めて困難で、事実上不可能である。それに反し、神経芽細胞腫は末梢交感神経細胞に由来するほぼ均一な神経細胞集団（腫瘍化してはいるが）から成り、均質に発現している神経関連遺伝子が得られる可能性が高く、また神経芽細胞腫は癌であるため、神経発生の未熟な段階で発現している重要な遺伝子が多いことが特徴として挙げられる。

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さらに、神経芽細胞腫は、予後のよいものと予後の不良なものとの臨床的、生物学的にはっきり分けられる。予後良好な神経芽細胞腫である癌細胞は増殖速度が極めて遅く、ある時点から自然退縮を始めることが特徴である。これまでの知見から、この自然退縮は、神経細胞の分化およびアポトーシス（神経細胞死）が起こっており、正常神経細胞の成熟段階で起こる分化とプログラム細胞死と非常によく似た現象が起こっているものであることが分かってきた。したがって、この腫瘍に発現している遺伝子を解析することは、神経の分化やアポトーシスに関連した重要な情報を入手できる可能性が極めて高い。

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さらに、予後不良な神経芽細胞腫は明らかに悪性増殖を続ける癌細胞からなる腫瘍である。したがって、神経細胞の増殖に関連した重要な遺伝子や、未分化な神経細胞で発現している遺伝子が多数存在する可能性が高い。つまり、予後良好な神経芽細胞腫で発現している遺伝子のプロファイルとは全く異なる遺伝子情報を入手する可能性が極めて高い。

一般的に神経細胞は、他の臓器由来の細胞と比較して、発現している遺伝子の種類が多いと言われている。神経芽細胞腫の細胞株（セルライン）は、予後不良の臨床組織由来であり、腫瘍化に伴い遺伝子発現のプロファイルが正常神経細胞と大きく変化しているものと考えられる。

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また、神経芽細胞腫は小児由来の腫瘍であることも一つの特徴であり、後天的な因子の影響が非常に少ない可能性が高く、癌発生のメカニズムの解析とともに発生学的な情報を入手できる可能性が高いことが予想される。また、さらに驚くべきことに本発明に係る遺伝子または遺伝子断片の中に、ある特定の細胞周期にのみ発現を増強する遺伝子が含まれており、このことから癌発生のメカニズムの解析および発生、分化に関する非常に有用な情報を入手できる可能性が高いことが予想される。

上記特徴を有し、上記情報を入手できる核酸は、ヒト神経芽細胞腫の臨床組織より得られ、配列表の配列番号1から366の塩基配列、またはその塩基配列の一部の塩基配列を有する。

さらに、ヒト神経芽細胞腫の予後良好なものと不良なものの臨床組織における遺伝子発現量を比較した結果、配列番号1から366の塩基配列を有する核酸の全てにおいて非常に顕著な差が認められた。すなわち、これらの核酸は予後良好なヒト神経芽細胞腫で発現が増強されていた。従って、配列番号1から366の塩基配列は、上記の有用な遺伝子情報以外に、それらの塩基配列を有するDNAおよび／またはRNAを検出することによって神経芽細胞腫の良不良を診断する腫瘍マーカーの情報としても利用可能である。

すなわち、本発明は、ヒト神経芽細胞腫およびそれに関連する様々な予後診断を以下の手段により実施可能とする。

(1) ハイブリダイゼーションに用いるプローブ

本発明において開示された塩基配列の一部、または全部からなる核酸（以下、本発明の核酸ともいう）をハイブリダイゼーションのプローブとして使用することによって少なくともヒト神経芽細胞腫において発現している遺伝子を検出することが可能である。また、本発明の核酸をハイブリダイゼーションのプローブとして使用し様々な腫瘍、正常組織における遺伝子発現を調べることで、遺伝子発現の分布を同定することも可能である。

本発明において開示された塩基配列の一部、または全部からなる核酸をハイブリダイゼーションのプローブとして使用する場合、ハイブリダイゼーション法自身については特に限定されない。好適な方法としては、例えばノザンハイブリダイゼーション、サザンハイブリダイゼーション、コロニーハイブリダイゼーション、ドットハイブリダイゼーション、Fluorescence in situ hybridization (FISH)、in situ hybridization (ISH)、DNAチップ法、マイクロアレイ法、などが挙げられる。

前記ハイブリダイゼーションの1つの応用例として、本発明の核酸をノザンハイブリダイゼーションのプローブとして用い、検定したサンプル中においてmRNAの長さを測定することや、遺伝子発現を定量的に検出することが可能である。

また、本発明の核酸をサザンハイブリダイゼーションのプローブとして用いる場合は、検定したサンプルのゲノムDNA中の、当該塩基配列の有無を検出することが可能である。

また、本発明において開示された塩基配列の一部、または全部からなる核酸をFluorescence in situ hybridization (FISH) のプローブとして用いることで、遺伝子の染色体上の位置を同定することも可能である。

また、本発明の核酸をin situ hybridization (ISH) のプローブとして用いることでその遺伝子の発現の組織分布を同定することも可能である。

本発明の核酸をハイブリダイゼーション用プローブとして使用する場合は、少なくとも40個の核酸残基の長さが必要であり、本発明に係る遺伝子配列のうち、40個以上の連続した残基を有する核酸が好ましく用いられる。さらに好ましくは、60個以上の核酸残基をもつものが用いられる。

当業者には、核酸プローブ技法は周知であり、個々の長さの本発明に係るプローブと目的とするポリヌクレオチドとの適当なハイブリダイズ条件は容易に決定される。種々の長さを含むプローブに対し至適であるハイブリダイズ条件を得るためのこのような操作は当業者では周知であり、例えばサンプルックら、「分子クローニング：実験手法(Molecular Cloning: A Laboratory Manual)、第2版、ワールドスプリングハーバー(1989)」が参照される。

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好ましくは、本発明のプローブは、容易に検出されるように標識される。検出可能な標識は、目視によって、または機器を用いるかのいずれかによって検出され得るいかなる種類、部分であってもよい。通常使用される検出可能な標識は、例えば、 ^{32}P 、 ^{14}C 、 ^{125}I 、 ^3H 、 ^{35}S 等の放射性標識である。ビオチン標識ヌクレオチドは、ニックトランスレーション、化学的および酵素的手段等によって、DNAまたはRNAに組み込むことができる。ビオチン標識されたプローブは、アビジン／ストレプトアビジン、蛍光標識剤、酵素、金コロイド複合体等などの標識手段を使用したハイブリダイゼーションの後検出される。核酸はタンパク質と結合させることによって標識されてもよい。また、放射性または蛍光ヒストン一本鎖結合タンパク質に架橋された核酸を使用してもよい。

(2) PCR法に用いるプライマー

遺伝子を検出する方法には他にも、本発明において開示された塩基配列の一部、または全部からなる核酸であるDNAをプライマーとしてPolymerase Chain Reaction (PCR) 法を用いることにより可能である。例えば、検定したいサンプルからRNAを抽出しRT-PCR法により遺伝子発現を半定量的に測定することが可能である。このような方法は当事者にとって周知の方法によって行われるが、例えばMolecular Cloning A LABORATORY MANUAL (T. Maniatis 著: Cold Spring Harbor Laboratory Press 社)、遺伝子病入門 (高久史麿 著: 南江堂) を参照して行うことができる。

本発明の核酸であるDNAをPCR用プライマーとして使用する場合は、10個から60個の塩基の長さが必要であり、本発明に係る遺伝子配列のうち、10個から60個の連続した塩基を有する核酸が好ましく用いられる。さらに好ましくは、15個から30個の塩基をもつものが用いられる。また一般的には、プライマー配列中のGC含量が40%から60%が好ましい。さらに、増幅に用いる2つのプライマー間のTm値に差がないことが望まれる。またプライマーの3'末端でアニールせず、プライマー内で2次構造をとらないことが望ましい。

(3) 核酸のスクリーニング

本発明において開示された塩基配列の一部、または全部からなる核酸を使用することによって様々な組織や細胞で発現している遺伝子発現の分布を検出することが可能である。例えば、本発明の核酸をハイブリダイゼーションのプローブ、またはPCRのプライマーとして使用することによって、遺伝子発現の分布を検出することが可能である。

またDNAチップ、マイクロアレイ等を用いても遺伝子発現の分布を検出することが可能である。すなわち本発明の核酸を直接チップ、アレイ上に張り付けことが出来る。そこに細胞から抽出したRNAを蛍光物質などでラベルし、ハイブリダイズさせ、その遺伝子がどの様な細胞で高発現しているかを解析することが可能である。またチップ、アレイ上に張り付けるDNAは本発明の核酸を用いたPCRの反応産物であっても良い。チップ、アレイ上に核酸を張り付ける方法の一例は、例えば、Heilerら米国特許第5605662号に記載されている。

上記技術を用い、本発明において開示された塩基配列の一部または全部からなる核酸のうち少なくとも一つを用いて診断薬として使用することが可能である。近年、ある疾患に罹患しやすいまたはしにくい、特定の薬剤が効くまたは効かないということが、個々人が持つ遺伝子情報によって支配されているということが明らかになりつつあるが、前記核酸を用いて製造したDNAチップやマイクロアレイ等を使用することにより、被験者における疾患と前記核酸との因果関係が明らかとなり、当該疾患の診断が可能となるばかりか、投与すべき薬剤の選択が可能となる。特に、DNAチップやマイクロアレイによる検出結果を投与すべき薬剤の選択の指標とするには、本発明の核酸のうち一つの核酸の発現量を検討するばかりでなく、二個以上の核酸の発現量を相対的に比較・検討し投与すべき薬剤の選択を行うことができ、より正確な判断が可能となる。ここで、前記疾患としては本発明にかかる核酸で診断可能な疾患であれば特に制限はないが、神経疾患であることが好ましく、神経芽細胞腫であることがより好ましい。

(4) DNAのクローニング

本発明において開示された塩基配列の一部、または全部からなる核酸を使用することによって少なくともヒト神経芽細胞腫において発現している遺伝子をクローニングすることが可能である。例えば、本発明の核酸をノザンハイブリダイゼーションのプローブ、コロニーハイブリダイゼーションのプローブまたはPCRのプライマーとして使用し、本発明において開示された塩基配列の一部、または全部を含む遺伝子をクローニングすることが可能である。クローニング可能な遺伝子としては特に、予後不良な神経芽細胞腫と予後良好な神経芽細胞腫で発現量に差がある遺伝子、他の組織や癌細胞での発現様式とは異なって発現している遺伝子、細胞周期依存的に発現している遺伝子、神経分化に伴って誘導される遺伝子、癌遺伝子または癌抑制遺伝子によって発現が制御される遺伝子等が挙げられる。

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(5) 腫瘍の予後同定の方法およびそのために使用可能な腫瘍マーカー

本発明において開示された塩基配列の一部、または全部からなる核酸をハイブリダイゼーションのプローブ、またはPCRのプライマーとして使用し、試料細胞中の遺伝子発現の増強の有無を調べることにより、予後同定が可能である。遺伝子発現の増強の有無を調べるには、例えば本発明により開示された塩基配列の任意の配列からなる核酸とハイブリダイズし得る核酸（プローブ）を使用する全ての方法が提供される。すなわち試料細胞中にプローブとハイブリダイズする核酸の量が増強する場合、予後が良好であると診断することが可能である。またPCRのプライマーとして使用する場合は、例えば、検定したいサンプルからRNAを抽出しRT-PCR法により遺伝子発現を半定量的に測定することが可能である。

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(6) アンチセンスオリゴヌクレオチド

本発明の別の実施態様では、本発明に係る塩基配列を有するアンチセンスオリゴヌクレオチドおよびアンチセンスオリゴヌクレオチドが提供される。本発明を実施する際に考慮されるように、本発明に係る塩基配列に対応するRNAに結合して、それによりRNAの合成を阻止することができるそのようなアンチセンスオリゴヌクレオチド、およびアンチセンスオリゴヌクレオチドは容易に調製できる。

(7) 遺伝子治療

本発明の別の態様では、遺伝子治療に用いられる治療用遺伝子が提供される。本発明を実施する際に考慮されるように、本発明に係る遺伝子を遺伝子運搬に使用されるベクターに導入して、任意の発現プロモーターにより導入遺伝子を発現させ、例えば癌の遺伝子治療に用いることができる。

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1. ベクター

導入されうるウイルスベクターは、DNAまたはRNAウイルスをもとに作製できる。MoMLVベクター、ヘルペスウイルスベクター、アデノウイルスベクター、AAVベクター、HIVベクター、SIVベクター、センダイウイルスベクター等のいかなるウイルスベクターであっても良い。また、ウイルスベクターの構成タンパク質群のうち1つ以上を、異種ウイルスの構成タンパク質に置換する、もしくは、遺伝子情報を構成する塩基配列のうち一部を異種ウイルスの塩基配列に置換する、シュードタイプ型のウイルスベクターも本発明に使用できる。例えば、HIVの外皮タンパク質であるEnvタンパク質を、小水痘性口内炎ウイルス(Vesicular stomatitis Virus: VSV)の外皮タンパク質であるVSV-Gタンパク質に置換したシュードタイプウイルスベクターが挙げられる(Naldini L等: Science 272 263-(1996))。さらに、治療効果を持つウイルスであれば、ヒト以外の宿主域を持つウイルスもウイルスベクターとして使用可能である。ウイルス以外のベクターとしてはリン酸カルシウムと核酸の複合体、リボソーム、カチオン脂質複合体、センダイウイルスリボソーム、ポリカチオンを主鎖とする高分子キャリアー等が使用可能である。さらに遺伝子導入系としてはエレクトロポレーション、遺伝子銃等も使用可能である。

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2. 発現プロモーター

さらに、薬物遺伝子に用いられる発現カセットは、標的細胞内で遺伝子を発現させることができるものであれば、特に制限されることなく何でも用いることができる。当業者はそ

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のような発現カセットを容易に選択することができる。好ましくは、動物由来の細胞内で遺伝子発現が可能な発現カセットであり、より好ましくは、哺乳類由来の細胞内で遺伝子発現が可能な発現カセットであり、特に好ましくは、ヒト由来の細胞内で遺伝子発現が可能な発現カセットである。発現カセットに用いられる遺伝子プロモーターは、例えばアデノウイルス、サイトメガロウイルス、ヒト免疫不全ウイルス、シミアンウイルス40、ラウス肉腫ウイルス、単純ヘルペスウイルス、マウス白血病ウイルス、シンビスウイルス、A型肝炎ウイルス、B型肝炎ウイルス、C型肝炎ウイルス、パピローマウイルス、ヒトT細胞白血病ウイルス、インフルエンザウイルス、日本脳炎ウイルス、JCウイルス、パルボウイルスB19、ポリオウイルス等のウイルス由来のプロモーター、アルブミン、SR α 、熱ショック蛋白、エロンゲーション因子等の哺乳類由来のプロモーター、CAGプロモーター等のキメラ型プロモーター、テトラサイクリン、ステロイド等によって発現が誘導されるプロモーターを含む。

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(実施例)

以下、実施例および製造例に基づいて本発明をより具体的に説明するが、本発明は以下の実施例に限定されるものではない。

(製造例1) ヒト神経芽細胞腫からのcDNAの構築

1. サンプル入手

ヒト神経芽細胞腫のサンプルを手術摘出直後に準無菌的に凍結し、その後 -80°C に保存した。

2. 予後良好サンプルの選別

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上記1で得られたサンプルについて予後の検定を以下の指標をもとに行った。

予後良好：

- ・病期1または2
- ・発症年齢が1歳未満
- ・手術後5年以上再発なく生存
- ・N-mycの増幅なし

予後不良：

- ・病期4
- ・発症年齢が1歳以上
- ・手術後3年以内に死亡
- ・N-myc増幅あり

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このうちN-myc増幅は以下のように確かめた。すなわち、実施例1にて得られた臨床組織を剃刀で細かく切断し、5mlのTENバッファー(50mM Tris-HCl (pH=8.0) / 1mM EDTA / 100mM NaCl)を加え良くホモジナイズした。この混合液に750 μl のSDS(10%)、125 μl のproteinase K(20mg/ml)を加え、軽く混和し 50°C で8時間放置した。その後、フェノール・クロロホルム処理を行い、最後にエタノール沈殿により、ゲノムDNAを精製した。5 μg の得られたゲノムDNAを制限酵素EcoRI(NEB社製)で完全に消化し、N-mycのプロープを用いてサザンハイブリダイゼーションによりN-myc増幅を調べた。

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3. 予後良好なヒト神経芽細胞腫の臨床組織からmRNAの調製

上記2において予後良好であると判断されたヒト神経芽細胞腫の臨床組織2-3gをTotal RNA Extraction Kit(QIGEN社製)を用いてトータルRNAを抽出した。抽出したトータルRNAをオリゴdTセルロースカラム(Collaborative社製)を用いてpoly A構造を有するmRNAのプールを精製した。

4. mRNAの脱リン酸化

上記3において調製した100-200 μg のmRNAのプールを67.3 μl の0.1%ジエチルピロカーボネート(DEPC)を含む蒸留滅菌水に溶解させ、20 μl の5 $\times$ BAPバッファー(Tris-HCl(500mM, pH=7.0) / メルカプトエタノール(50mM))、2.7 μl のRNasin(40unit/ μl :Promega

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社製)、 $10\mu\text{l}$ のBAP ($0.25\text{unit}/\mu\text{l}$ 、バクテリア由来アルカリフォスファターゼ：宝酒造社製)を加えた。この混合液を 37°C で1時間反応させ、mRNAの5'末端の脱リン酸化処理を行った。その後、フェノール・クロロホルム処理を2回行い、最後にエタノール沈殿により、脱リン酸化mRNAのプールを精製した。

5. 脱リン酸化mRNAの脱キャップ処理

上記4において調製した脱リン酸化mRNAのプールの全量を $75.3\mu\text{l}$ の 0.1% DEPCを含む蒸留滅菌水に溶解させ $20\mu\text{l}$ の $5\times\text{TAP}$ バッファー (酢酸ナトリウム (250mM 、 $\text{pH}=5.5$) /メルカプトエタノール (50mM)、EDTA (5mM 、 $\text{pH}=8.0$)、 $2.7\mu\text{l}$ のRNasin ($40\text{units}/\mu\text{l}$)、 $2\mu\text{l}$ のTAP (Tobacco acid pyrophosphatase: $20\text{units}/\mu\text{l}$))を加えた。この混合液を 37°C で1時間反応させ、脱リン酸化mRNAの5'末端の脱キャップ処理を行った。この際キャップ構造を持たない不完全長の脱リン酸化mRNAは脱キャップ処理されず5'末端は脱リン酸化された状態に留まる。その後、フェノール・クロロホルム処理、エタノール沈殿により、脱キャップmRNAのプールを精製した。

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6. オリゴキャップmRNAの調製

上記5において調製した脱キャップmRNAのプールの全量を $11\mu\text{l}$ の 0.1% DEPCを含む蒸留滅菌水に溶解させ、 $4\mu\text{l}$ の5'-オリゴRNA (5'-AGCAUCGAGUCGGCCUUGGCCUACUGG-3': $100\text{ng}/\mu\text{l}$)、 $10\mu\text{l}$ の $10\times\text{ligation}$ バッファー (Tris-HCl (500mM 、 $\text{pH}=7.0$) /メルカプトエタノール (100mM))、 $10\mu\text{l}$ の塩化マグネシウム (50mM)、 $2.5\mu\text{l}$ のATP (24mM)、 $2.5\mu\text{l}$ のRNasin ($40\text{units}/\mu\text{l}$)、 $10\mu\text{l}$ のT4 RNA ligase ($25\text{units}/\mu\text{l}$: 宝酒造社製)、 $50\mu\text{l}$ のポリエチレングリコール ($50\%w/v$ 、PEG8000: シグマ社製)を加えた。この混合液を 20°C で3時間反応させ、脱キャップmRNAの5'末端に5'-オリゴRNAを連結した。この際キャップ構造を持たない不完全長の脱リン酸化mRNAは、5'-オリゴRNAが連結されない。その後、フェノール・クロロホルム処理、エタノール沈殿により、オリゴキャップmRNAのプールを精製した。

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7. オリゴキャップmRNAからのDNA除去

上記6において調製したオリゴキャップmRNAのプールを $70.3\mu\text{l}$ の 0.1% DEPCを含む蒸留滅菌水に溶解させ $4\mu\text{l}$ のTris-HCl (1M 、 $\text{pH}=7.0$)、 $5.0\mu\text{l}$ のDTT (0.1M)、 $16\mu\text{l}$ の塩化マグネシウム (50mM)、 $2.7\mu\text{l}$ のRNasin ($40\text{units}/\mu\text{l}$)、 $2\mu\text{l}$ のDNase I ($5\text{units}/\mu\text{l}$: 宝酒造社製)を加えた。この混合液を 37°C で10分間反応させ、余分なDNAを分解した。その後、フェノール・クロロホルム処理、エタノール沈殿、カラム精製 (S-400HR: ファルマシアバイオテック社製)により、DNA(-)オリゴキャップmRNAのプールを精製した。

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8. 1st strand cDNAの調製

上記7において調製したDNA(-)オリゴキャップmRNAのプールをSuper Script II (ライフテックオリエンタル社製キット)を用いて逆転写し、1st strand cDNAのプールを得た。DNA(-)オリゴキャップmRNAのプールを $21\mu\text{l}$ の滅菌蒸留水に溶解させ、 $10\mu\text{l}$ の $10\times\text{First Strand}$ バッファー (キット付属品)、 $8\mu\text{l}$ のdNTP mix (5mM 、キット付属品)、 $6\mu\text{l}$ のDTT (0.1M 、キット付属品)、 $2.5\mu\text{l}$ のオリゴ-dTアダプタープライマー ($5\text{pmol}/\mu\text{l}$ 、5'-GCGGCTGAAGACGGCCTATGTGGCCTTT TTTTTTTTTTTTTT-3')、 $2.0\mu\text{l}$ のRNasin ($40\text{units}/\mu\text{l}$)、 $2\mu\text{l}$ のSuper Script II RTase (キット付属品)を加えた。この混合液を 42°C で3時間反応させ、逆転写反応を行った。その後、フェノール・クロロホルム処理、アルカリ処理、中和処理にて全てのRNAを分解し、エタノール沈殿で精製した。

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9. 2nd strand cDNAの調製

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上記8で調製した1st strand cDNAのプールをGene Amp (パーキンエルマー社製キット)を用いてPCRにて増幅を行った。1st strand cDNAのプールを52.4 μ lの滅菌蒸留水に溶解させ、30 μ lの3.3 $\times$ Reactionバッファー(キット付属品)、8 μ lのdNTP mix (2.5mM、キット付属品)、4.4 μ lの酢酸マグネシウム (25mM、キット付属品)、1.6 μ lのプライマーF (10 pmol/ μ l、5'-AGCATCGAGTCGGCCTTGTTG-3')、1.6 μ lのプライマーR (10 pmol/ μ l、5'-GCGCTGAAGACGGCCTATGT-3')、2 μ lのrTth (キット付属品)を加えた。この混合液に、100 μ lのミネラルオイルを静かに加え重層した。この反応液を94 $^{\circ}$ Cで5分間変性させた後、94 $^{\circ}$ C、1分間・52 $^{\circ}$ C、1分間・72 $^{\circ}$ C、10分間を1サイクルとして12サイクル繰り返し、さらに72 $^{\circ}$ Cで10分間放置しPCR反応を行った。その後、フェノール・クロロホルム処理、エタノール沈殿で精製し2nd strand cDNAのプールを得た。

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10. 2nd strand cDNAのSfiI処理

上記9で調製した2nd strand cDNAのプールを87 μ lの滅菌蒸留水に溶解させ、10 $\times$ NEBバッファー (NEB社製)、100 $\times$ BSA (ウシ血清アルブミン、NEB社製)、2 μ lのSfiI (制限酵素、20 units/ μ l、NEB社製)を加えた。この混合液を50 $^{\circ}$ Cで一晩反応させ、SfiIによる制限酵素処理を行った。その後、フェノール・クロロホルム処理、エタノール沈殿で精製し両末端がSfiI処理されたcDNAのプールを得た。

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11. SfiI処理されたcDNAのサイズ分画

上記10で調製したSfiI処理されたcDNAのプールを1%のアガロースゲルで電気泳動し、2 kb以上の分画をGene clean II (Bio 101社製)を用いて精製した。精製したcDNAのプールは100 μ lの滅菌蒸留水に溶解させ、37 $^{\circ}$ Cで6時間放置した。その後、フェノール・クロロホルム処理、エタノール沈殿で精製し長鎖cDNAのプールを得た。

12. cDNAライブラリーの調製

上記11で調製した長鎖cDNAのプールをDNA Ligation kit ver. 1 (宝酒造社製キット)を用いてクローニングベクターであるpME18S-FL3 (東京大学医科学研究所 菅野純夫先生より供与)にライゲーションを行った。長鎖cDNAのプールを8 μ lの滅菌蒸留水に溶解させ、あらかじめ制限酵素DraIIIで処理された1 μ lのpME18S-FL3、80 μ lのSolution A (キット付属品)、10 μ lのSolution B (キット付属品)を加え、16 $^{\circ}$ Cで3時間反応させた。その後、フェノール・クロロホルム処理、エタノール沈殿で精製しcDNAライブラリーを得た。

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(実施例1) 大腸菌へのトランスフォーメーション

製造例1で調製したcDNAライブラリーを大腸菌 (TOP-10: Invitrogen社製) にトランスフォーメーションした。cDNAライブラリーを10 μ lの滅菌蒸留水に溶解し、TOP-10に混合した。その後、氷上にて30分間、40 $^{\circ}$ Cで1分間、氷上で5分間インキュベートした。500 μ lのSOB培地を加え、37 $^{\circ}$ Cで60分間振盪培養した。アンピシリンを含む寒天培地上に適量づつ播種し、37 $^{\circ}$ Cで一昼夜培養して、大腸菌クローンを得た。

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得られた寒天培地上の大腸菌クローンを、爪楊枝にて拾い上げ、96穴プレートに準備した120 μ lのLB培地中に懸濁させた。この96穴プレートを37 $^{\circ}$ Cで一晩静置し、大腸菌の培養を行った。その後60%グリセロール溶液を72 μ l加え、-20 $^{\circ}$ Cで保存した (グリセロールストック)。

(実施例2) 塩基配列の決定

1. プラスミドの調製

実施例1で調製した10 μ lのグリセロールストックを15 mlの遠心チューブに移し、3 mlのLB培地、50 μ g/mlのアンピシリン加え、37 $^{\circ}$ Cで一晩振盪し大腸菌の培

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養を行った。その後、Q I A G E N社のQ I A p r e p S p i n M i n i p r e p K i tを用いて大腸菌からプラスミドDNAを抽出、精製した。

2. 両末端シークエンスの解析

上記1で調製したプラスミドDNAをDNA Sequencing Kit (ABI社製キット)を用いて両末端のシークエンスを決定した。600ngのプラスミドDNA、8μlのプレミックス(キット付属品)、3.2pmolのプライマーを混合し滅菌蒸留水で合計20μlになるように調製した。この混合液を96℃で2分間変性させた後、96℃、10秒間・50℃、5秒間・60℃、4分間を1サイクルとして25サイクル繰り返し反応を行った。その後エタノール沈殿で精製した。変性条件下でポリアクリルアミドゲルにて電気泳動を行い、配列解析を行った。解析にはABI 377 (ABI社製)を用いた。

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(実施例3) データベースを用いたホモロジー検索

実施例2で両末端シークエンスを解析したサンプルについてインターネットを介したDNA配列のホモロジー検索を行った。ホモロジー検索にはNCBI (National Center of Biotechnology Information USA)のBLASTを用いた。

(実施例4) 半定量的PCRによる予後良好・不良ヒト神経芽細胞腫での遺伝子発現量の測定

実施例3で得られた、遺伝子の一部から、PCRプライマーを合成し、ヒト神経芽細胞腫の予後良好・不良の臨床組織で発現量を比較定量した。実施例3で示した方法でヒト神経芽細胞腫の臨床組織よりmRNAを抽出し、rTaq (宝酒造社製)を用いてPCR反応を行った。5μlの滅菌蒸留水、2μlのmRNA、1μlの10×rTaqバッファー、1μlの2mM dNTPs、各々0.5μlの合成プライマー・セット、0.5μlのrTaqを混合した。この混合液を95℃で2分間変性させた後、95℃、15秒間・55℃、15秒間・72℃、20秒間を1サイクルとして35サイクル繰り返し、さらに72℃で6分間放置しPCR反応を行った。この反応液を1%のアガロースゲルで電気泳動した。半定量的PCRによる予後良好・不良ヒト神経芽細胞腫での遺伝子発現量の測定結果の一例を図1に示す。なお、図1中の各レーンの説明は以下のとおりである。レーン1~8: 予後良好神経芽細胞腫臨床サンプルにおけるnb1a-4183 (配列表の配列番号154、155)の発現

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レーン9~16: 予後不良神経芽細胞腫臨床サンプルにおけるnb1a-4183 (配列表の配列番号154、155)の発現

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レーン17~24: 予後良好神経芽細胞腫臨床サンプルにおけるGAPDHの発現

レーン25~32: 予後不良神経芽細胞腫臨床サンプルにおけるGAPDHの発現。

この結果、配列番号1から366の塩基配列において予後良好ヒト神経芽細胞腫でのみ発現量が増強する核酸が確認された。

(実施例5) マウス上頰神経節細胞におけるNGF調節アポトーシスにより発現量が変化する遺伝子群

ヒト神経芽細胞で抗分裂剤(例えば、ネオカルジノスタチン)により誘導されるアポトーシスに対して、NGF(神経成長因子)が保護作用を呈することが知られている(Cortazzo MH, J. Neurosci, 16, 3895-3899 (1996))。この作用は、p75にNGFが結合して、アポトーシスを抑制するためであると考えられている。また、臨床段階での神経芽腫の退縮(予後良性化)は、腫瘍細胞でのアポトーシスと関連があるとの指摘もある。

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そこで、神経芽腫細胞中でのアポトーシス関連遺伝子を同定する目的で、マウス上頰神経節細胞におけるNGF調節アポトーシスにより発現量が変化する遺伝子群を調べた。

まず、0~1日齢C57BL/6Jマウス7~10匹から上頰神経節細胞(SCGニューロン)を分離した。24ウェル細胞培養用プレートに1ウェル当たり約1~2x10⁴個の細胞を7ウェル培養することにした。さらに、約1~2x10⁴個の細胞からRNAを回収した(0日対照)。

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前記7ウェルの細胞にNGF (5 mg/ml) を添加した培養液を5日間、培養した後、1ウェルの細胞からRNAを回収した(+NGF、5日後)。6ウェルの各3ウェルの培養液をNGF (5 mg/ml) または抗NGF抗体(1%)を添加した培養液で、交換して、それぞれ12、24、48時間培養した。一部のサンプルについては、NGFを添加した場合と抗NGF抗体を添加した場合の形態学的な相違を確認するために、細胞を電子顕微鏡で観察した。この結果を図2に示す。NGFを添加した細胞では、NGF誘導分化が見られたが、抗NGF抗体を添加した細胞ではアポトーシスが見られた。抗NGF抗体によるNGF除去(NGF depletion)の結果、アポトーシスが誘導されたことが明らかである。上記の12、24、48時間培養後の各ウェルの細胞からRNAを回収した。これらのサンプルを+NGF 12 hrs、+NGF 24 hrs、+NGF 48 hrs、-NGF 12 hrs、-NGF 24 hrs、および-NGF 48 hrsと表示することにする。

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上記のようにして、約160匹のマウスから得た各処理細胞のRNAをエタノール沈殿法で濃縮して、1 µgのRNAから逆転写酵素を用いて、cDNAを合成した。これらcDNAをテンプレートとして、PCRにより遺伝子の発現量の比較を行った。ここで使用したプライマーは、ヒト神経芽細胞で見出される遺伝子を基に検索したマウスの相同遺伝子から作成した。発現比較に使用したプライマーの塩基配列を比較のターゲットとなる遺伝子の識別番号(ID)と共に表にまとめたものが、表1である。

表 1

プライマー名	プライマー配列	プライマー名	プライマー配列
nbla00031m-F3	TCAGAAGGCTTCG AGACTG	nbla00031m-R3	GCAGATATCTTGTCAAAGG T
nbla00100m-f	GGTGAGCCCTAAC ATCCACA	nbla00100m-r	AGCCCGTAAGCCATCAATC
nbla00115m-F3	ATCATGGTGAAAG GCACGTC	nbla00115m-R3	CATAATTTCCACGTGTTGTG
nbla00116m-F3	AGAGGTTAGCCCT GAGACAG	nbla00116m-R3	TCTATGCTTCCAGCAGGTA C
nbla00124m-F3	CCTTTATATTAGAA CGTGGC	nbla00124m-R3	TAGGTTTTTCAGGACTTGG
nbla00144m-F	GCAGGATAGCAAC CTTGACA	nbla00144m-R	ATGCATGCTTTCGTGTGTTG
nbla00150m-2f	CACCCTGGAGAAA CACAACTC	nbla00150m-2r	ATGCCGTCCATCTTCATCAC
nbla00170m-F3	AACATGAATCTGT GGGTGAC	nbla00170m-R3	TCAATCGCTTGACCTTCCTC
nbla00204m-F3	AAGCATTGAAGTA ACAACCG	nbla00204m-R3	TGCATTTGACATATGAGAG C
nbla00225m-F2	TATTAGAACGTGG CCCTCCA	nbla00225m-R2	TCGGGACTAGCAGGACAGA A
nbla00301m-F3	TAAACCAGCAACC CTAACAG	nbla00301m-R3	ACTAGAATCAGACCTGCCT T
nbla00315m-F	AACTGGCAGCAAA GAAGGTC	nbla00315m-R	AGCAGAAGCAGGTCAGTAA GG
nbla00402m-F2	TCACCCAGAATGA GACATGG	nbla00402m-R2	GTCAGAAGGTGACACGGTG A
nbla00433m-F3	CCCAAGGAGGTAA GAGCCTG	nbla00433m-R3	AAGCGCTGGAGCTTGTCCG T
nbla00437m-F3	AGAACGGTGTAAT TCAGAGA	nbla00437m-R3	GTCATCAGCAAGCTCGAAT A
nbla00537m-F3	TTTATATTAGAACG TGGCCC	nbla00537m-R3	AGGACTTGGTAGCTTCTCG G
nbla00551m-F	AATTTTAAAAAGG CAGGATATACAAC	nbla00551m-R	AAGGAGAGGTAGCATTTTA TGTGC

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ori00660m-F	GGCATCCTCATCTC CTCAGA	ori00660m-R	TCTGAAGCAGTTCGAAGCA C
nbla00761m-f	TGGTTCAAACCTCTT CCCTCCT	nbla00761m-r	CTTCCACCTGTGCGTTTTCT
nbla00831m-F3	TGGGAAATCCACC TGCATC	nbla00831m-R3	ACCAGCTCACCTGAATGT G
nbla00908m-2f	CGTGGCAACACCTT TTTATTG	nbla00908m-2r	ACACGGCATGGGTTTGT
nbla02874m-f	TGAGGAGTTGAAT GCTGACCT	nbla02874m-r	CATCGGGGTTAATGCTCTT G
nbla03086m-f	ACTTGCTCATGGTG CTGTCTC	nbla03086m-r	CGCAGAGCCTGGTAATCTT C
nbla03113m-F	CCTCAAGAACCAG ACCAAGC	nbla03113m-R	CTGATGCTGAGGAGCTGAC A
nbla03199m-f	GGCCACCAGAGAG GTAATG	nbla03199m-r	GTGCTGACCTAAGACCCAA AG
nbla03267m-f	CTGCCTTTGAGATG GTGATG	nbla03267m-r	TGTAGTGCTTTGCATTGTTG G
nbla03646m-f	TACCTCTTCGGCTG GATGG	nbla03646m-r	GCTTGGGCAGGATGAATG
nbla03755m-F	AGCCGATGAAGTG TCTGCTT	nbla03755m-R	RAGCCACAAAAGCAGGTTA GG
nbla03771m-f	GCAGCAGATATAG GGACACAGA	nbla03771m-r	CACGCATAAATGGCTACAC C
nbla03777m-F3	AGATTATTCACCTG TAAGC	nbla03777m-R3	TTTTCCACATGTCCAGCACC
nbla03831m-f	GTGACCAGCAAGG AAACACA	nbla03831m-r	GAGGATTCAGCCACGAACA
nbla03862m-F	GCATGGAGGAAAC CATTAGG	nbla03862m-R	TGGCTCTTCAACCAAACCT T
nbla04021m-F2	ATGACCTGGCACT AGGCTTG	nbla04021m-R2	AACACATCTGCTGGCTTCT G
nbla04137m-f	ACGACGACACTGA CAACCAC	nbla04137m-r	GCTTGACCTCCGACTCATCT
nbla04196m-f	CACCCATCTGTGTC TGTGGT	nbla04196m-r	CGTGCTGACGATGATGTTG
nbla04261m-2f	GTGTTTGGCACTAC ATCACCA	nbla04261m-2r	CCGGGTTTTCCATTTTCAC
nbla04300m-f	TACAGTGGGAACT GCGTTTG	nbla04300m-r	CAGGGTTCGTATGCAGGAG

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nbla10058m-f	AGCCATTACAGGT GGCAAGA	nbla10058m-r	GTTGGGTCGATCTTAGGAG GTAG
nbla10070m-F3	TAACAACCTCTAGC ACCATC	nbla10070m-R3	TACTTAGCAGAACAGAAGA G
nbla10071m-f	GTGGACACCAAGA ATGCAAG	nbla10071m-r	CAGCCAACTGTGGTAAGAA GG
nbla10120m-F	CAGTGCAGCCTTGG AAGTGT	nbla10120m-R	TCAAAAGCTGCGTGTGTCT C
nbla10143m-F3	TTAGTGAGTACAC GAGCTGG	nbla10143m-R3	ACTTAACCCAGACTGACCA C
nbla10283m-F3	AGATGTTTAAGGG CAAACC	nbla10283m-R3	TGGAGCCTCTTGGATCTC
nbla10300m-F3	AACATCCTGGTGG AACAGC	nbla10300m-R3	CTCTATAGTAACGACCAAA C
nbla10314m-f	GCTTGAGGACAGT GAAAACCA	nbla10314m-r	TGGAGTGAGAGGATGGGAA G
nbla10317m-2f	GCAGGGGACACAG GACTCTAC	nbla10317m-2r	AAGCTCCTTCTGGCTCAAC A
nbla10329m-F	CCGAGATCTTCTGC CTTCAT	nbla10329m-R	TCCTTGCCGTCTCAAACCTCT
nbla10363m-F3	ATCTCTCTAGTGCC ATGAC	nbla10363m-R3	AGTCTTGCTAAGACTTTCA G
nbla10383m-f	CAGTGCGGTTGTGG TCTATCT	nbla10383m-r	TGAGGCGTTGACTTTCTGG
nbla10388m-F	TTCAGCAGGTCCTA GCCAAG	nbla10388m-R	RTGGAAGCTGCTGAAGAAA CA
nbla10457m-F	GCCTTTCTTTGTCT GGAACG	nbla10457m-R	GGGTGAAGCAATTTACAG G
nbla10485m-F	AAATGGCAGTTTG ACTGTGG	nbla10485m-R	TTGGCTGAGTTCTCCCTCAT
nbla10527m-F3	ATTAATCCTGCACT CTTACG	nbla10527m-R3	AGTTCCATTCTACAGCAA G
nbla10535m-F	TTGGTCGTGAGGTG GATTCT	nbla10535m-R	ATCTTGCCAGCCACAGACT T
nbla10545m-F	AGAGTCACCTGCG ACCCTTA	nbla10545m-R	AGCTCTAGCAGCCAGCACA T
nbla10677m-f	CATAATCTTCTCCG GCTTCATC	nbla10677m-r	GTCTGGTATTTCCGTGAGGT TT
nbla10687m-F3	ATCTCCGATCGACT CACTGC	nbla10687m-R3	TGGCTTTACTGGTCATACA G
nbla10696m-f	TGATTCTCCAAGGC AAGGT	nbla10696m-r	GATTTCCCCATTGACTGCT

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nbla10988m-2f	AGCCTTTGCTACCCTC TTCC	nbla10988m-2r	GGCGAAACACTCCTCTCGT
nbla11030m-F3	AACCTCGTAAAAACC ATGGC	nbla11030m-R3	AGCAGTGACTTGAGCATTG
nbla11042m-2f	CGACACCTCTCATTGC ACAC	nbla11042m-2r	TCCGTCTCAAATCCACACAC
nbla11051m-f	AGCACAATTCCCCAG ACAC	nbla11051m-r	CTGTAGCCCTTACTGTTTGACC
nbla11189m-F	CTGTGTTCTGATGCCA ATGC	nbla11189m-R	TGCAACTTTCTCCACCAAGA
nbla11589m-F2	GGAGGTAGCCAAGAT GATCG	nbla11589m-R2	CTGGCCATCCTAGAGGAGAA
nbla11882m-F	AATTTTAAAAAGGCA GGATATACAAC	nbla11882m-R	AAGGAGAGGTAGCATTTTATGT GC
nbla11895m-F3	AATCTTCCTCCCAACC CATG	nbla11895m-R3	TGACCCTGCTGAAGGAAGCG
nbla20001m-F2	CCCTGAATGTTGAACG AGTG	nbla20001m-R2	TGCACATTGAAGAGGCAAAAC
nbla20019m-F1	CCGAGGTCAACATTTG TTCC	nbla20019m-R1	GAGCAGAGCACAGACAGTGG
nbla20125m-F1	CTGGAATCATCCAGG CTTTG	nbla20125m-R1	GCGTCCAGGATAACAGCACT
nbla20134m-F1	GTCTTCAAGCAGCGA CAGTG	nbla20134m-R1	CCTTCAGGGTCTGGTTGATG
nbla20146m-F1	GGGACCACTAACCAG CTGAA	nbla20146m-R1	AAATGTCTGACCCCTCCTCA
nbla20181m-F1	CCAGGATGGAGTAGC CAAGA	nbla20181m-R1	GCCAGTGATCTCCAGGTTTG
nbla20182m-F1	ACTGGGGAGGAATGG CTAGT	nbla20182m-R1	CTGGCTGGAGGAAAAGGAC
nbla20211m-F1	CTGAGGTGCTGATGAT CCTG	nbla20211m-R1	CCAAACTCCTGCTCTTCTCG
nbla20231m-F1	AGCAGTTTGGTGCTGT TGGT	nbla20231m-R1	GCTTGTTCCATGGTTGGACT
nbla20250m-F2	ACGAGGACCACAGGA CTCAA	nbla20250m-R2	TGGGCTCCATAGTTTGTTCC
nbla20268m-F1	ATTCTCTCTGGAGGCG ATGA	nbla20268m-R1	TTTCTCACAGCTCCCTCTC
nbla20378m-F2	TTTAGCTGTTTGCAA TAAGATGT	nbla20378m-R2	AATGCAGTGTGTGGGATGTG
nbla20421m-F1	TCCGACATGATGGTTC TCCT	nbla20421m-R1	AGATCCAGGAGTCACCCAAA
nbla20487m-F3	CACAGGTGTCAAAGC ACGTT	nbla20487m-R3	TGTAGCACTCGCTGTTGCTC

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nbla20511m-F1	TCCTGAAGCCTTCTTG CCTA	nbla20511m-R1	GCTTGTGGCAACCAGAAAGT
nbla20541m-F1	AGGTGGGAGTCGACC TTTCT	nbla20541m-R1	GCATCCTTCAACTTGGTCCT
nbla20638m-F1	GCAGCAAGGAAGAGG ACAAG	nbla20638m-R1	AGCAATCTTCGTCTGGGAAG
nbla20688m-F1	AGAAGGACCTCCTCC CAAAG	nbla20688m-R1	TGCAACAGTCCTCTTCCTTG
nbla20709m-F2	ATTAGTTGGGACCTGC CTTG	nbla20709m-R2	GCCCATTTTCTTCAGCAGAG
nbla20765m-F1	AGAGCAGCTCAGCTA CCACA	nbla20765m-R1	GAAGGCAACTTTGGTGTGG
nbla20798m-F1	AAACGGGTACAGGAT GGAGA	nbla20798m-R1	CAACTGGAGGTCGGAGGATA
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nbla24986m-F1	CAGAGCAGTAACCGT GACCA	nbla24986m-R1	ACCAGGACGGACACTGTTGT

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遺伝子発現量の変化が観察された遺伝子について、以下のような結果が得られた。NGF depletion (−NGF) によって、発現量の減少が見られた遺伝子は、nbla-03646 (配列番号121) (−NGF12hrs)、nbla-03771 (配列番号125) (−NGF12hrs)、nbla-03831 (配列番号132) (−NGF24hrs)、nbla-04300 (配列番号165) (−NGF24hrs)、nbla-10120 (配列番号171) (−NGF48hrs)、nbla-10329 (配列番号187) (−NGF24hrs)、nbla-10363 (配列番号188) (−NGF12hrs)、nbla-10677 (配列番号229) (−NGF12hrs)、nbla-10696 (配列番号232) (−NGF12hrs)、nbla-11051 (配列番号273) (−NGF24hrs)、nbla-11189 (配列番号281) (−NGF24hrs) であった。また、NGF depletion (−NGF) によって、発現量の増加が見られた遺伝子は、nbla-03862 (配列番号133) (−NGF24hrs) と nbla-10485 (配列番号210) (−NGF24hrs) であった。さらに、NGFの添加により、発現量の増加が見られた遺伝子は、nbla-10143 (配列番号174) (+NGF) であった。

これら特定の遺伝子を増幅するのに使用したプライマー・セットを表2に示す。

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表 2

プライマー名	プライマー配列	プライマー名	プライマー配列
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nbla03831m-f	GTGACCAGCAAGGAAACACA	nbla03831m-r	GAGGATTCAGCCACGAACA
nbla03862m-F	GCATGGAGGAAACCATTAGG	nbla03862m-R	TGGCTCTCAACCAAACCTT
nbla04300m-f	TACACTGGGAACTGCGTTTG	nbla04300m-f	CAGGGTTCGTATGCAGGAG
nbla10120m-F	CAGTGCAGCCTTGAAGTGT	nbla10120m-R	TCAAAAGCTGCGTGTGTCTC
nbla10143m-F3	CAGTGCAGCCTTGAAGTGT	nbla10143m-R3	TCAAAAGCTGCGTGTGTCTC
nbla10329m-F	CCGAGATCTTCTGCCTTCAT	nbla10329m-R	TCCTTGCCGTCTCAAACCTCT
nbla10363m-F3	ATCTCTCTAGTGCCATGAC	nbla10363m-R3	AGTCTTGCTAAGACTTTTCTAG
nbla10485m-F	AAATGGCAGTTTGACTGTGG	nbla10485m-R	TTGGCTGAGTTCTCCCTCAT
nbla10677m-f	CATAATCTTCTCCGGCTTCATC	nbla10677m-r	GTCTGGTATTTCCGTGAGGTTT
nbla10696m-f	TGATTCTCCAAGGCAAGGT	nbla10696m-r	GATTTCCCATTTGACTGCT
nbla11051m-f	AGCACAATCCCCAGACAC	nbla11051m-r	CTGTAGCCCTTACTGTTTGACC
nbla11189m-F	CTGTGTTCTGATGCCAATGC	nbla11189m-R	TGCAACTTCTCCACCAAGA

上記のように、NGF depletionによって、上頸神経節細胞は死滅（アポトーシス）することが知られているので、その際発現量の変化を示す遺伝子は、アポトーシスの機構に密接に関連している。

産業上の利用可能性

以上説明したように、本発明によれば、神経芽細胞腫の予後良不良に関係する遺伝子配列を明らかにし、その遺伝子情報の提供および予後良不良に関する診断が可能となる。

【配列表】

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<212> DNA

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<211> 899

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<213> Homo sapiens

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<220>

<223> nbla-00185-f

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<211> 887

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<213> Homo sapiens

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<223> nbla-00185-r1

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<210> 16

<211> 864

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00226-f

<400> 16

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 gctgtgcctc totgaaaagn aagntnaaaa tccgggtcca aaancgttnc atgaatggan 780
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<210> 17.

<211> 872

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-00226-r1

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<400> 17

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 cgttgnctcn ccnaantgcc cgggtccctg gaaaactggg gcaactatac ngngctcaa 720
 ggtaaccaan ggccccgaag gcngttggtg gtanaactcc cngnngnat cctggaatcc 780
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<211> 928

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00258-f

<400> 18

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ggtaccgtgg tggctctgcc gggggccggg gcttcttccg tgggggccga ngacggggcc 540
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<210> 19

<211> 931

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00258-r1

<400> 19

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 gtccgttcga tntcagagaa gtgagtgggg caaccacttc atggggccaa cccactaaa 420
 aaaaaaagg caacgcaacc aaaaactaaa aaaaaacca aaaaccacac aacccccgat 480
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<211> 880

<212> DNA

<213> Homo sapiens

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<223> nbla-00312-f

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 aacatgtggg tgatgtaaac ttcaaattgg gagatgttcc gacccccact cccatataag 600
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atangttgggt gcaacttaca atcccganc t ggtaatccaa tantttgggt ttaacaatgg 780
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<210> 21

<211> 887

<212> DNA

<213> Homo sapiens

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<223> nb1a-00312-r1

<400> 21

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<211> 1740

<212> DNA

<213> Homo sapiens

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<223> nbla-00315

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<400> 22

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<211> 843

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<213> Homo sapiens

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<211> 836

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00417-r1

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40

tccaanccat aaggggccaan taaatgtttg nggtanccga tacatctaaa attattttaa 660
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<211> 844

<212> DNA

<213> Homo sapiens

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<223> nbla-00428-f

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<400> 25

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gtgtgtctat cattaaaact tgtattccaa tctgtccaac ntaaggngat naagatatta 720
anangnancc ctgatttaat ttccagtatt cagtagtaaa atttcctgtc cactggtgan 780
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844

<210> 26

<211> 842

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00428-r1

<400> 26

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<210> 27

<211> 879

<212> DNA

<213> Homo sapiens

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<400> 27

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 ccntaaaaaa gtggggatgt ntacatttgt taacaatgcc atgaaagcaa tttccttccc 780
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<211> 866

<212> DNA

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<213> Homo sapiens

<220>

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<400> 28

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<211> 847

<212> DNA

<213> Homo sapiens

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<223> nbla-00460-f

<400> 29

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<210> 30

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<211> 862

<212> DNA

<213> Homo sapiens

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<223> nbla-00460-r1

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<400> 30

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<210> 31

<211> 859

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00478-f

<400> 31

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<210> 32

<211> 863

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-00478-rl

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<400> 32

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<210> 33

<211> 831

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00483-f

<400> 33

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tccctaacct gcaacatttta tgtcgcatgt caatccgaag agtgatgccc acccaagaag 600
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<211> 874

<212> DNA

<213> Homo sapiens

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<223> nbla-00483-r1

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40

tggntaatcc tcatcaattc tccaaaacct caccaattin acaatcagca aggnnttgcac 840
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<210> 35

<211> 879

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00491-f

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<210> 36

<211> 892

<212> DNA

<213> Homo sapiens

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<211> 885

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<212> DNA

<213> Homo sapiens

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<223> nbla-00494-f

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<211> 890

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<211> 862

<212> DNA

<213> Homo sapiens

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<211> 888

<212> DNA

<213> Homo sapiens

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 gagggacaca tgggagggca tttctgtnga accatgacag tcaccatgat ggcagcgcaa 720
 tataagttac tgacaaatat tcaaacatct ctagataaat aatccaagtc ccaaatttca 780
 aatcactgag taatgacttt ccgnggtcaa acnttagttt ggaataaaat tgcaataatt 840
 aatatccaa aatggtaatg gcacggtgac tgcngttgaa nccatttt 888

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<210> 41

<211> 923

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00536-f

<400> 41

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 tcttttagga cagtgtcct ctcttcttcc cgggtcgtg ccagcaagta cggagagagg 180
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ctcagaattt gtgtcgtgtc tctaaaccag ataatggag agtactaaaa gcattgttgc 300
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 cantttnnng nagncaeng tgnntnnnt aantngagg tangnnntgc tannchnatn 840
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 gcgttannnn atnngcccca gtg 923

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<210> 42

<211> 877

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-00536-r1

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<400> 42

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 tcgggggcag gacttatgtg cagagctagg tccgtggctc tggtaaggc actgccttgg 180
 gtcactctgt gctggcccag gcnngctcca tgcttggtg ctggataanc cagtctctt 240
 ctgaccccca aagcaatcca atgggtgggg aaactcagga tctctctcan cccanagatc 300
 tgactgatga ggcatangtt ttanctatt tgtaaaaaa tgaaagtatc tcatactacc 360

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attgacacag ccaggtggga nggggtcact ggngaaacgc naactgnnnnc tgcccatggg 420
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 tgggtgtanaa cctgagattc antctttcaa gtgggaagaa gcactgganc atggnacant 540
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 ccctaggaan ttttcagang gncattttnn tgggtangnc ttngcnaanc aancggntt 780
 ttntcctggg cttttcggca antaacttt ngntccnnac ctttntgatn ttatncnatt 840
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<210> 43

<211> 2105

<212> DNA

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<213> Homo sapiens

<220>

<223> nbla-00538

<400> 43

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 tgggtgggctc tagaatggtt ttggagctcg agatcttcat gggtttagact tgcgtggtcag 180
 acccaggagc acctgtggct cacaccttct gttccccctcc tggcctgtgc agaatgtaaa 240
 cagcagactc atactcaatg ggcactacag gccttatcag acgttttata caagcctgga 300
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 gagctgagcc gaaatataag aataatatat ttgtgcttcg agttgggtgt tctttcagtg 480
 taatgcatgc agtggtcaca acccagttac tcataatatt tggattgtat ttgttcgtag 540

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40

atatgcccag aagactagag aattagtgtt atataccata tagaacttac tgtcagtcaa 600
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 tgttgccaga tatgcatatt ggaatcccat ctacccatag ttcctctgaa ggtgattttg 780
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 gtaaaattac agtattccaa aggttgaagt tctgctgttt tgttataatg cctgatacac 2040
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2105

<210> 44

<211> 863

<212> DNA

<213> Homo sapiens

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<223> nbla-00543-f

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<400> 44

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 ggaccgaagt ctggacatca atgccttttag gaaagaaaaa gatgacaagt ggaaaagagg 180
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 catggatttg ttgggccttg atgctcctgt ggcctgctcc attgcaaata gtaagaccag 360
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 gttcatggct ccgctcaga tggatatccc acaagcctac ccagcttcc ccgggggttac 660
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 aaggaacttc tggcatgcag goatcaatga tgggtgtgcc gaatggaatg atgaccancc 780
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<210> 45

<211> 874

<212> DNA

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<213> Homo sapiens

<220>

<223> nb1a-00543-r1

<400> 45

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aatcttttaa gcaaagtttt ctgtcttgaa ttttcaagt gggtgaacaa tgactgagag	180	
gaaagctgtc ccggccctct gcctcgtaca cctgggaacg gtggggaaac agagcaccct	240	
ggatacacag gcatgaaaga gtgatcagea gaccgggaga agggaaggga gaaagggagt	300	
tatcaatgac atggcgtttt ttaaaccata agaaaaacac aacagtttta ggctgctgat	360	
aaattaattc ctctctgttg taaacctaaa actaaacaaa aacaaaaata ccagagcag	420	
atggggaaag ggtgggaggc ggaacacaca ctcatacaca cacacacact cagcacaca	480	20
cacaagcagt acacgttaag ctctgcattc ccactatagg ggttggtcct ccagccacgg	540	
agccagattc tactgaaggc aagggggaag cnggaaggga tgtgtgtcng tgtgtgggat	600	
nttcanaaag tccttggggc taataaaggn tgactgaanc aacaagtcct gaaaccccaa	660	
attcaaaaag caactcaagg cgaananagt antaattttg ctcatcctca tangangggg	720	
cttgccctca cccaaggcta aggtcaaact gacccaagac ccanggagta ttaatttgat	780	
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<210> 46

<211> 898

<212> DNA

<213> Homo sapiens

<220>

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<223> nbla-00547-f

<400> 46

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 gtgagagacg gaccgagacc ggagatgttt tcaagcccg gctccggogg ctttacaggc 120
 ggctgcagcg gcgacgaaga caacgacagc gacggctacg ccgaagcact cgttccgggg 180
 gtgaagcctc ctgcgccggc cttgcctcgg atccaggatg agaagactga taaaagaaga 240
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 gaagctggaa ataagaaatc cacaceaatt agcattcctt ttggttatec actctctgag 660
 cgaaaacaga tggcacttcn tatgcagatg acagcaagag acaacaatcc agattccaca 720
 ccaaatcacc catcacaac aacgcctgn caaaagaaaa ctccaattc ttcatctcga 780
 cagaangnta aagntaataa aagaaatgaa cgtgggggaaa ctcttacac atggntgcaa 840
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20

<210> 47

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<211> 840

<212> DNA

<213> Homo sapiens

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<223> nbla-00547-r1

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<400> 47

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 ttttcaccaa attcnaagtg gaaatctttc tgatgtttgc tttttcctta agcttacaag 180
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 tttcctttta ctttgcttc cgtttgtgtc acctgaaaca catatccttt ttgggttgca 600
 aacaaaattg angttcaagg atgacggaca tgaagtanaa anctttatcc gaaanagctt 660
 ctcacaaaac ttcagtactt ganataagac ccaaatggnt ttggttttac ctttaaagga 720
 ttaagntaaa anggttcncc cgnttgcaaa aaangagttt ttcngetca agncaattc 780
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<210> 48

<211> 871

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00567-f

<400> 48

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 ctaacgtttc taacaggggt gttaatgaca ttagcagcaa gagctatgag aaataacttt 180
 agacattatt tcattgaacc ttcccaactg aaattatttt atgatgttat aacatggata 240

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gtaactcaag tagcaataag ttacacagtt gtgccatttg tgcttcttte tataaaacca 300
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 caaacaatgt ttgcaatcag aatcaagaaa tagcctcgag acattcatca ctaaagcagt 540
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<210> 49

20

<211> 901

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-00567-r1

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<400> 49

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 agttatcaat cttttcctgg gtaggggttag ctttatcagc cggaaaacac ttacttttag 420

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ataaataaaa atagattagg atattttccaa acttcaaaaa agacacatgc ccatttttgc 480
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 atagcataag ccaaatgggt gccttgnct taactcaatt tgcaaattta taatggctcc 600
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 taaggnaaaa agttaaaant agnttcaa acataggtcaa acaaangngg aatcaaaaag 720
 gnttgccct cccctcccaa aaggnaaggn ggnanaaac tggggntnaa ggcantccaa 780
 aggcctggga ncaaaatcca atcctttggn ntttaanncc cgggggcaac aaaantccnc 840
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10

<210> 50

<211> 881

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00569-f

<400> 50

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40

gictgaacct ttaaaacatc tgtagagca aaattaaaaa gagcatttgg tagtaatcca 600
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 aaaaaagtta catgtcantt ggngaaaata cgttatccag aanttgtgca taaantgatg 720
 ccaaaaaagg canttcaaca ttgtggacaa ngggagacac tntttaaant cccgaaagaa 780
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<210> 51

<211> 884

<212> DNA

<213> Homo sapiens

<220>

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<223> nbla-00569-r1

<400> 51

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 cagaagaaaa gggaaataaa attttccccc caaacacat aagaaccact tactggcact 420
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 tcaacaanca ncccttaaatt tccaantttt aaatngntgn tcaaacgaca ncaataaaaag 660
 tccttccccc ttaaataagg gggnaatnna tgctacaagn atcaaattcc ttggtnaagg 720

30

40

acaaagnant ccctcaagga aatcaanttc ccgggccctg gtaanccaac aagnnaatcc 780
 ntttatngga caaccggtta acaaattaaa aatgntggca attnttncca ggggacaant 840
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<210> 52

<211> 793

<212> DNA

<213> Homo sapiens

10

<220>

<223> nbla-00639-f

<400> 52

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 tgtatatatt ttaactgcac tggtagattg aacatgtcag tgcgatgcc actggaccaa 120
 cagagcattt tgtggctata gggtagctga taataatgtc ttgatttttc tttgggattc 180
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 gttatcngaa tgtgnnnnan tnanthcatg angaaaatta acccgatnc acaaaaacag 600
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 ttttngaga aagncannnn anangtctt tctnaatngg nanaatccca annnnnncca 720
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20

30

40

<210> 53

<211> 882

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-00666f

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<400> 53

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<213> Homo sapiens

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<223> nbla-00666r1

<400> 54

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<400> 55

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<212> DNA

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<223> nbla-00724-r1

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791

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<211> 484

<212> DNA

<213> Homo sapiens

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<220>

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<400> 59

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<211> 473

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00757-f

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<210> 62

<211> 487

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-00757-r1

<400> 62

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<400> 63

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<211> 815

<212> DNA

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<211> 867

<212> DNA

<213> Homo sapiens

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<223> nbla-00784-f

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<211> 2146

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<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-02969-r1

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<213> Homo sapiens

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<223> nbla-03037-r1

<400> 80

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<211> 1895

<212> DNA

<213> Homo sapiens

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<223> nbla-03066

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<212> DNA

<213> Homo sapiens

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<223> nbla-03096-f

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<212> DNA

<213> Homo sapiens

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<211> 836

<212> DNA

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<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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<211> 734

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<220>

<223> nbla-03199

<400> 91

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<211> 2295

<212> DNA

<213> Homo sapiens

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<220>

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<223> nbla-03213-f

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<212> DNA

<213> Homo sapiens

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<223> nbla-03219

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<223> nbla-03298-r1

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<211> 730

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<213> Homo sapiens

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<211> 736

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<213> Homo sapiens

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<223> nbla-03421-f

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<213> Homo sapiens

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<223> nbla-03421-r1

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<211> 732

<212> DNA

<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-03439-r1

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<211> 2231

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-03461

<400> 106

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<211> 737

<212> DNA

<213> Homo sapiens

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<223> nbla-03477-f

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<211> 750

<212> DNA

<213> Homo sapiens

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<223> nbla-03477-r1

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<211> 2342

<212> DNA

<213> Homo sapiens

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<223> nbla-03526

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<211> 739

<212> DNA

<213> Homo sapiens

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<223> nbla-03539-f

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<211> 747

<212> DNA

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<223> nbla-03539-r1

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<400> 111

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747

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<211> 725

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-03587-f

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<211> 734

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<223> nbla-03587-r1

<400> 113

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<211> 805

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<213> Homo sapiens

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<223> nbla-03598-f

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 thaaancann ccnnaanaan nacccaaanc canncaccgc aaaannnnnt cccanennn 720
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<211> 752

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-03598-r1

<400> 115

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<210> 116

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<211> 3168

<212> DNA

<213> Homo sapiens

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<223> nbla-03614

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<400> 116

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<212> DNA

<213> Homo sapiens

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<220>

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<211> 778

<212> DNA

<213> Homo sapiens

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<211> 757

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-03636-f

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<210> 120

<211> 751

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-03636-r1

<400> 120

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20

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<211> 4219

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<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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<211> 761

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<213> Homo sapiens

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<223> nbla-03949-r1

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<211> 693

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<213> Homo sapiens

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<211> 4125

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<213> Homo sapiens

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<213> Homo sapiens

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<211> 747

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<213> Homo sapiens

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<223> nbla-04183-f

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<211> 793

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<213> Homo sapiens

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<211> 2753

<212> DNA

<213> Homo sapiens

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<223> nbla-04196

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<212> DNA

<213> Homo sapiens

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<220>

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<211> 769

<212> DNA

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<223> nbla-04219-r1

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<400> 157

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<211> 768

<212> DNA

<213> Homo sapiens

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<223> nbla-04241-f

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<400> 158

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<210> 159

<211> 3553

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<213> Homo sapiens

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<211> 1971

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<213> Homo sapiens

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<220>

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<213> Homo sapiens

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<211> 784

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10

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<211> 793

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<213> Homo sapiens

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<211> 3028

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-04300

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<213> Homo sapiens

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<211> 781

<212> DNA

<213> Homo sapiens

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<211> 1475

<212> DNA

<213> Homo sapiens

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<400> 168

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<211> 1557

<212> DNA

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<220>

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<210> 170

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<211> 1783

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<213> Homo sapiens

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1783

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<223> nbla-10120

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<211> 801

<212> DNA

<213> Homo sapiens

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<213> Homo sapiens

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<211> 1880

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<213> Homo sapiens

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<223> nbla-10242

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<211> 811

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<400> 222

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<211> 825

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<213> Homo sapiens

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<223> nbla-10624-f

<400> 223

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<223> nbla-10624-r1

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<220>

<223> nbla-10635

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<211> 838

<212> DNA

<213> Homo sapiens

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<220>

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 aatnnntnta ntntnctc cncntnanat aatnctatnt nnttatatac accattttat 780

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<213> Homo sapiens

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<210> 259

<211> 815

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<212> DNA

<213> Homo sapiens

<220>

<223> nbla-10934-ri

<400> 259

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 tggggagatg aggagaatag ggcaggcagc aacagggcaa catgcatttt tcaagagtgt 240
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 acttccctaa agtacaagg catttcccta gtagtcttgg taccagtaac aatatgatta 420
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 gatatatatt ttttcatttc tccagtgttt tttattttta taaatatgca cctagtttcc 600
 taccagttta aaacacacac gaaacactgn ttttgagcac catgattgcc ttacagtagc 660
 ctctttggga ctncaatcct agttcaagga ggggagtcct aatttctatt ctgacttgnt 720
 tcnttcccca aatnatgtta gnggttgccc aaatccatca attttnagat gccacaaaag 780
 ggttttgggc naangggcca atnataattt tgant 815

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<210> 260

<211> 835

<212> DNA

<213> Homo sapiens

<220>

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<223> nbla-10939-f

<400> 260

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 gtgccttctt tggcaagatg tgtgaatctg tgacttgtcc angancagaa tgtacctaag 780
 tcaagatcca taacgcatgt tggtaaggtc acttggacca naactttgtg aacan 835

<210> 261

<211> 824

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-10939-r1

<400> 261

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atagctttga agtgagaact tacagcccat ctgaacagtg aggtttgtac ccaggcatac 120
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 tgcagcatg tgcacctgc gcagacattt acagacagc aactcagtct ttctcacttg 240
 gacaaacctgt tgtgcaaaaa cactaggaat cttgaagtga gggagctctg tccacagctc 300
 ccgtaatggc agggaggaga gcagagccca atgaaccttg agtgaaatta agtgctcgaa 360
 taaccacata attaaattgt gcagatcagc agtcctagtc tgggaatttaa aactgtcaa 420
 cggggcatat tgggaaagat atttatatat atatatatac atacacacac acacacacac 480
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 aaaacctgna ccatcagtta acagaaccag gtggcttcag atgcactatg aaaccttct 720
 atttaaaca aagngcttnt tcatgtctac ctgggggacc tttgntggg cattgaacag 780
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<210> 262

<211> 816

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-10946-f

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<400> 262

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 agctgagaaa tggagtgaag ttttgtaaag tacaaaactt tctgagatt ttgtgcatcc 180
 acctaaaag attcagacat gaactaatgt tttccaccaa aatcagtacc catgtttcat 240
 ttccgctaga aggcttggat cttcagccat ttcttgctaa ggatagtcca gctcaaattg 300

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tgacatatga tcttctgtca gtcatttggc atcatggaac tgcaagtagt ggacactata 360
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 aaagctgggt atattgaaga cctgggtttg atgctgcctc anaacatttg ggataaccta 720
 tatagcaggt atgggtggagg ccacatgcaa ncatctgnac atttggcacc ctggccaatt 780
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<210> 263

<211> 826

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-10946-r1

<400> 263

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 aaacaaaaac aaagccatca tgaatgctat tcaacatcct caatgtaate cagtatgttt 180
 ttgtacttgg aatatagtta aacttttgac attacataat caagcaaata gcagtgcata 240
 ctatattatt caaaaagact ttatctatct catttaaaaa atcaagttgc aagtggcctc 300
 agctttatca acaatcgtag tgacacatte cacacttcat gctctcaaaa taaaagtgct 360
 cctaaaacta actctaagtt ttttagtcac tgacattaat actaaccagg ttacaggaat 420
 tgaagtttaa cattgtacaa tataagcggc aataagttac tgatatctgc tgacaaattc 480
 cactcaaact aaatatatcc ttgatcattc aaaaagacat ttgttaaatt ttaataagct 540

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agtttagtgna tatgctgcag tgcaaaaaaa gtgacaccct agtttcctga gctttaggaa 600
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 gcaaaactnt ncaattaaat tgggcttttg ggagaactga aagactgggc agaagtcang 720
 ggggatttan gacaatggaa aaagnttcaa gncaaagttt ttccacactn ccaaaggttc 780
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<210> 264

<211> 1735

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-10949

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<400> 264

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 cattatitttg agaattaaaa cacctacttg tttccagatg ccacctgcct ctgtgagtgt 240
 gggcatgagt atgtgtgtgt tgccggaggtg ggtgaggag ttggaggagt tttgttacac 300
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 ttaaaaataga ggtgatgttc ctaaaaataa ttgtactcta tagcacataa gatgaattaa 720
 ttgactagaa acataggtaa ctctgagcct gaagtatgac tggaagtttg gcctgttatg 780

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tatgtctgta tgaaatagtg tacattaatt aaataagggt tggggttaat agaaaaataa 840
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<210> 265

<211> 725

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-10967-r1

<400> 265

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ggattaacat ttttgggtgtt aaaattgtta aacatcatgc ttactgcaca tcaactctcc 180
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<210> 266

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<211> 710

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-10977-f

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<400> 266

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 gaaaaacagg attagaaaca cacttaaaaa gaacacacat ttagagtctc tcttcctcct 180
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ttgctattgc agtgcagagc ccactgcaac tgctaggact gaaagcagag gctgggtgcc 480
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 atagtgcata ggaacaagta aagccagggt gcttgacgtg tgtgtgtgtg tgtgtgtgtg 660
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<210> 267

<211> 717

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-10977-r1

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<400> 267

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<210> 268

<211> 1979

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-10993

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<400> 268

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<210> 269

<211> 727

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-10999-f

<400> 269

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<210> 270

<211> 731

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-10999-rl

<400> 270

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ctataacaaa tattgacacg aggggggaca aaagcatggt tgaaatgtat tagaatcaag 600
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<210> 271

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<211> 1834

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11030

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<400> 271

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<223> nbla-11067-f

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<223> nbla-11067-r1

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<213> Homo sapiens

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<223> nbla-11090-f

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<223> nbla-11090-r1

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<212> DNA

<213> Homo sapiens

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<223> nbla-11110

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<212> DNA

<213> Homo sapiens

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<223> nbla-11176-r1

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<211> 2035

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<213> Homo sapiens

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<223> nbla-11189

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<400> 281

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 aagtgggtact tctaaatcat aaaagttggg gaaagagacc tttaaaatct tgtgggtgtg 1920
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<210> 282

<211> 726

<212> DNA

<213> Homo sapiens

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<223> nbla-11201-f

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<400> 282

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 attcagctac ccttggctac ccaagtagtc taaacatttt atctttatca ggcacatata 180
 cagtcctggtt ctctgtagcc aaatttagat acccatgaca ttatatctaat gatttcaaac 240
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 atcttcaaag gccgtgacca ctgagcaagg gtcagtggca cctctcccca ctgtctctctc 480
 accaggtagc ttcattccat ccattcatgc attcaactac ccttcgtgtt gtccagcact 540
 atgtatagtg ctgcagaaac aagaaggata tagtgcttgc cttcaaagac ctccaaagtg 600
 gggagatagt ctggatgaca ggtgctgtta tagaagtgtg cccccggatg ctataggagc 660
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ctcctn

726

<210> 283

<211> 727

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11201-r1

<400> 283

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 ccgagtagct gggattacag gcacatgccg ctaccgccca gctaatttat gtatttttag 180
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 gcccgcttca gcctcccaaa gtgctgggat tacaggcgtg agccaccaag ccagccaag 300
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 tactttatta aggcactggg acctatagct ttcacttctt taatatctca tattgggggt 660
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<210> 284

<211> 704

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<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11212-f

<400> 284

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 gatcacacaa aatgtttagg gagatgttcc cgtgggtgta tcctcatggt aacaacgaca 180
 aaaaatgccg gttgtctttg ttctcttttc actattccta acatgtgtac atgatagctt 240
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 ctcagtattt tgaaaattga gattacttca gagccttagc cacacctaaa atacctccta 480
 gttaatagtg gtataaatgc ctcttcaata cgttttccag aatccaaagc attttggttt 540
 atccaggacc canggcagca cagctgtcac caagcaggag agttaaggat tcaccatgag 600
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<210> 285

<211> 720

<212> DNA

<213> Homo sapiens

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<223> nbla-11234-f

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<400> 285

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 agaggttagac aggtcacctt actacctcct cctcgtggc agggctcaaa ctgaaagagt 180
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 ggtgtaatct gctttcctgt ctgtagtgcc tgcgtgttgg aaagggtgag ttagaatatg 660
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<210> 286

<211> 758

<212> DNA

<213> Homo sapiens

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<223> nbla-11234-r1

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<400> 286

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 caggctcccc tagtccccac caccttgccc catccatcat gacgtggtgg ggaggggttg 180
 agacccatct ttaaaagtgg gggacagcag ggacaaagga tgggaaactg gaggaacagg 240
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aagggtgggg tcagtctagt ctctcaaagg ctgggtgaaaa gttttttttc acctattcct 360
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 ttctctctta tgagtaattt ggaacccaça gtccagcttg agggatatcc tgatatgcac 480
 aatgaatata aaccacggcg gatgcagatt tcaggatcat gctggggcct ggcccatttg 540
 ggtgggagga gctttctgtc aggagttgct gggtgcgggg taccgtgtgt gcccttcant 600
 gtggtgaagt gagaacatgt gaatgtgtgt gcattgtacca tccaaaccgt aagaggcaac 660
 ttgggtcang aggacccac ttnttccttt atgggacaac agtggantgg gaanaaacng 720
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<210> 287

<211> 714

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11269-f

<400> 287

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 cagaaggcat ccactagcg atcagctgac attcctaact gaaggctgca atgtgttgct 240
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 gcgggccacg gcgatcccag gcctgagcgg cggctaccca gtcagttcag agccaggccc 480
 tncactaccg gaacagagag cgctttgcac gatcaaatca gcatttttg ttacacgaca 540
 gatccatgag catgagcagg agaacgaagt tgcgggaaca agatgtcang gtataagccg 600

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gatgccgagg ccagcaccag aagcagctga tcgcctggag aacaagctga angctgagat 660
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<210> 288

<211> 715

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11269-r1

<400> 288

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 aatcttctgc tcaagggtgt ctctgcgcag agacactctc tgctctagct tctggagctc 240
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 ccatttctg ctggagctgt agcctcaagg cctggcattc tgcttcttga gctcatcta 360
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 aaagtncatg acatgccttt ctgtgcagnt ctctttttnn gctctttatt gnacttcaan 660
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<210> 289

<211> 746

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<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11270-f

<400> 289

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atgatgtata tgtccgggca tatatgtaca gcttccgtgc acatatgtgc caacctgtat 660
gtgccaacac ggcggtacat atatgtcgnc tccgtcattt attcttcccc antgggggtgg 720
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<210> 290

<211> 761

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11270-r1

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<400> 290

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 ggtaataaaa ttcacatggt cttccctaaa ctgaaggcca cttttgcata aaacagagca 480
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 ttcattctat tctttctacc taaacagctt ctgctctcct ctgggggtgg ggtggcaaag 660
 aaanggcaaa tcacccacaa nggggattaa agttacagaa atatttagac atcattcaca 720
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10

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<210> 291

<211> 731

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11279-f

<400> 291

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gaaaaagtta atcttgatat aaatttgtgt ttgataaata tcctctcagt gttttatctt 240
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 ggaaactttt ctcttcaaat ttgaaagctg nttctgtttt cattttacat tattattcag 420
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 ctgtaagcat tttaggtaga ttgccctaaa gggttatggga gggcatgagg gaacacttct 540
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 gacagaacaa tcactttaac accctttctn acaataatat aaaaatatta aaagaacata 660
 ggcaggcttt ttttaaattt ggcttttttc tttccttttt tcaaattgac ttttatagn 720
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<210> 292

<211> 734

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11279-r1

<400> 292

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 tgatggaatt aaatacaccc cttctcagt ctccagaaag gatactccta acccacccta 480

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40

aaatatgata aaataaatgc ccctattctg aactgtttaa ctgggaaata aaccatgccc 540
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 gaaagcaatt ctttggatat aagttaggag cttttaagaa gtatataaac tcttttange 660
 atctagagta gagacncaa gaggatttct tcataaataa tgatttance agngcaatgg 720
 ntggttgcca tggc 734

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<210> 293

<211> 761

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11307-f

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<400> 293

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 acctggnggg taaccattcc tttctggatt ctgaanagnc cctaccttg gataacctata 720
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<210> 294

<211> 751

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11307-rl

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<400> 294

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 nccactttta angcnggaaa tcngacota aatnccactt ggtactactt ttgggttaaa 660
 gcacaagctt nttaaaactc ctaaaattat tggataactg naaaanaggg taattgnncc 720
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20

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<210> 295

<211> 735

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11378-f

<400> 295

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aagaagcaca attttgattt tgatgagtca ctttctgtaa acaatcttgg tctatcttta 180	
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<210> 296

<211> 737

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11378-r1

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<400> 296

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<211> 723

<212> DNA

<213> Homo sapiens

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<211> 718

<212> DNA

<213> Homo sapiens

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 agaatagga gagacagcat gggttactga aaccatttat tcagaaagca gaggaaaggc 540

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gaaaggacac tgaatanac atttttaggt ttttatagga agttccaaat ggcagcagtg 600
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<211> 726

<212> DNA

<213> Homo sapiens

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<223> nbla-11469-f

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<211> 3121

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<213> Homo sapiens

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<223> nbla-11473

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<211> 718

<212> DNA

<213> Homo sapiens

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<210> 302

<211> 703

<212> DNA

<213> Homo sapiens

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<223> nbla-11483-r1

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<400> 302

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<211> 2650

<212> DNA

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<223> nbla-11485

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<211> 743

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<213> Homo sapiens

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<210> 305

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<220>

<223> nbla-11519-r1

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<211> 731

<212> DNA

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<220>

<223> nbla-11530-f

<400> 306

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40

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<211> 766

<212> DNA

<213> Homo sapiens

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<223> nbla-11567-f

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<400> 307

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 tagccataaa accatctcaa tttttgagga acgagccac atnctttata tgtccttaga 600
 aaagctaaag gttatcgatg atcctgaagt gtaccttoga agatctgncc ntataaacia 660
 tttgatgaaa aggatccatg gagaaattat catgcnnгаа tactggngct ttccttgctg 720
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<210> 308

<211> 772

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11568-f

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<400> 308

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 accacacagg gagagtgacc attctagtct aaataacaaa ttttgaatg gatgtggaga 180
 aatatcagtt tcagaaatga atgaaaagtt gacaactctg tgttatagga agtataatga 240
 tgtctctgat ctttgcaaat tagaaaataa acaatattgt aggtggtcca agaaccaaga 300
 tgacagtttt acaatttgca ggaaacttac atatccttta gaaactcttt gcaatagtcc 360
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 acagagctat tctattgggt atgaaggtag ctatgatgcc tctgctgac tctttgatga 480
 tattgctaaa gaaatggaca ttgcaactga gattaccaa aaatcacagg atattttggt 540
 aaaatgggga acatcttttg cagaaagtca cccttcagag tctgattttt cactgagatc 600
 actttctgaa gacttcattc agccttnaca aaaattatcc ttgcaaagcc tatctgactc 660
 tangcattca agacatgctc ttcaacacct nattttcaat cagattcang aatatnattt 720
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<211> 771

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11568-rl

<400> 309

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 taaaaccctg gggaaaacaa tggctttata ttttaaagg cacagcagct cccagcagag 240
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 tcttggtgga tcaagggaaa tgattggcag caaggcattt ncctaganct atgncttggn 720
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<210> 310

<211> 753

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11600-f

<400> 310

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 caactggttt atgtacttga gaagcangtg ttaacttctt cagttacaaa tgagaaaacc 720
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<210> 311

<211> 751

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11600-r1

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<400> 311

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 accactacac ccggotagtt ttttgatatt ttagtagaga tggggattca ctatgttgac 240
 cagactcgtc ttgaactcct gacctcgtga tccaccccac tcggattccc agagtgcctg 300

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gattacaggc atgagccacc gcgcccagcc agcattgtat actcttccaa aggtacttct 360
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 tcattatcaa gtggcccaaa gctataaaac ttatggntaa catatttagc ctcaggaaac 480
 ttagcttttag gaaagtcaat ctggagagac atgaatgaaa aatgattgcc tgctgaaaat 540
 taagcantta taatggttta cgataataac agaaaaactt ctatggcatt tactatgtgc 600
 catttgaaac ttaccacaaa ctaactcatt taatccactc aagaatccgc ttaagtaggt 660
 actggtggtta ttctttncat tttncanaag angagaaact gaggggtaag acattcagna 720
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<210> 312

<211> 736

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11618-f

<400> 312

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 tgctttgtcc ttcgtatga caactacaaa aagcaagcca gtggggactc ctgtggggcc 360
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 atcggatact ttgtaggcct gctcactgct actgtggcgt ctgcattca ccgggcgcgc 480
 agccgcctt ctctatttgg tgccatttac ttatttgcca ctctcagca tggectattt 540
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cgattcctgg aagtatgatg gatcacgtgg aaagtgacca gatggccctc atggnccttt 660
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<210> 313

<211> 733

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11618-r1

<400> 313

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 aaaaaaaaaa tacaaaaaaaa tagaaaatag aaaaatgtat ttacaagtag tacgttacta 180
 tgatgagaaa gcacaaagac acctgctgct ataatacatg cattggctcg agaagaaact 240
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 cttcatcccc aatttgctac actgatcaaa accaagtaag ggctcctgaa gtccatgagt 360
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 gaaggacagc agcttatcca tatacaagga agccacagta aactgctoga catgctcaaa 480
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<210> 314

<211> 2036

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11652

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<400> 314

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 gctgcaagga cagtgaggag ggagatggcc atgggtgcctt ggtccccaga gtgacggcat 180
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aggccecgaca cttccaggct gtgtggcctt gatcaagtta gtttcccttt cgtgattcag 1260
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 aggctgggca cagtggctca tgcctgtaat tccccttgg gaagcccagt tccatctcta 1980
 aaaaaaaaaa aaaaaaaggc cacatgtgct cgagctgcag gtcgcggcgc ctagac 2036

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<210> 315

<211> 728

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11674-f

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 aaacaacttc agtgactgtg gtgtatatgt attgcagtat gtagagagct tttttgagaa 180
 tccaattctc agttttgaac tacctatgaa ttgggcaaac tggtttctc caccaagaat 240

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gagaacaaaa agagaagaaa tccgaaacat aattctgaag ctacaggaag atcagagcaa 300
 agagaaaaga aagcataagg acacttactc aacagaagca cctttaggcg aaggaacaga 360
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 ttttttcaact tggtaggaag cttttggtat gnatttctgg taatagtacc taaaattgca 660
 acttctaacc caaatnaaaa ggaaaatatt tataggagga aatgattaat tggatatcta 720
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<210> 316

<211> 729

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11674-r1

<400> 316

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 aacaaaaaat gactaaaata tgtttcaagt ctcaaagttc atttgaaaga agtatacacc 540

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atttcatttg aaaaataatg aagttttctgc agatatatgc aagatcccta tatgtgctat 600
 aaatctgnca tggaaccat caaatittag ttattttaaa ggggaattca aagatttttg 660
 ctggattatg caactggtgg aatacgtact ttacatgtaa aaaataatta ccttggagna 720
 actcaagcc 729

<210> 317

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<211> 776

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-11679-f

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<400> 317

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 anngaaattn gctgtactca ggnagatgga tttcantctt ttgnccttc ngnantgann 720
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30

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<210> 318

<211> 753

<212> DNA

<213> Homo sapiens

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<223> nbla-11679-rl

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<400> 318

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 aagattggaa ctttggtttt tggagtctgc aagtgtctgt gtcantgaan aagaacact 720
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20

30

<210> 319

<211> 799

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11753-r1

<400> 319

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<210> 320

<211> 759

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11763-f

<400> 320

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 ataggactct ttttttggtt ttaagagtaa aatagaagaa gatctaagaa atacaaatac 480
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 aagatgttaa gaaagancag ttctttctaan tactctggca gatcgggtgg ctgaaagagg 720
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10

20

<210> 321

<211> 759

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11763-r

<400> 321

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 aggtatcaag tgatgacaga cacttacgtg aggtatacaa tcaattaaag actggaaatt 180
 ggggtattat catgtaagac atttgagagg ccaaaagtca tataatttta agatacttca 240

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tgatgttaca gtaataatc atctatataa caattatttt gacataagt gcatatcaga 300
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 aaaaaggngc ttctatctta gnagattctt ttaaatactt anctatatt naaaagtagt 660
 cnttacatac ttaagaaatc accgtaatct aaaaaantaa angcagtttc ttgnaatga 720
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<210> 322

<211> 778

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-11767-f

<400> 322

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 cgtcacccgg gaagcagtgt ggggacttca gtggggtgtc ccttagtgga gaacacctt 180
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<223> nb1a-11767-r1

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<211> 792

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<223> nbla-11822-r1

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<223> nbla-11860-f

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<212> DNA

<213> Homo sapiens

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<213> Homo sapiens

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<211> 1907

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<211> 812

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<213> Homo sapiens

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<223> nbla-12022-r1

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<212> DNA

<213> Homo sapiens

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<211> 1007

<212> DNA

<213> Homo sapiens

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<223> nbla-12074-r1

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<400> 347

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nntcctttcc aatttttnatn ttanccaacc cncctttttn ttcthnngga gnggggnnaa 900
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<211> 963

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<213> Homo sapiens

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<220>

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<211> 977

<212> DNA

<213> Homo sapiens

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<223> nbla-12075-r1

<400> 349

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977

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<211> 2698

<212> DNA

<213> Homo sapiens

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<223> nb1a-12092

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<210> 351

<211> 968

<212> DNA

<213> Homo sapiens

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<223> nbla-12112-f

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<400> 351

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968

<210> 352

<211> 913

<212> DNA

<213> Homo sapiens

<220>

<223> nb1a-12112-r1

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<400> 352

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<210> 353

<211> 1133

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<212> DNA

<213> Homo sapiens

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<223> nbla-12119-f

<400> 353

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<210> 354

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<211> 952

<212> DNA

<213> Homo sapiens

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<223> nbla-12119-r1

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<400> 354

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<210> 355

<211> 957

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<213> Homo sapiens

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<211> 891

<212> DNA

<213> Homo sapiens

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<210> 357

<211> 978

<212> DNA

<213> Homo sapiens

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<223> nbla-12124-f

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10

20

<210> 358

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<211> 916

<212> DNA

<213> Homo sapiens

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<223> nbla-12124-r1

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<400> 358

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 cacacacaaa ggcagggtatt aggttactga gttgggttct tcaccaaata gcaaagccag 660
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 cttttttttt accccaagcc ggttttcttg ctgtgnnttg ganggaaggg ccagggccna 840
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<210> 359

<211> 970

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-12137-f

<400> 359

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 tgggaaaaca gtggnttaat tottaaatca agtacanggc taatttcctt ttgnaatct 900
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<210> 360

<211> 950

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-12137-r1

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acatgaagct aggtgtggaa ttttccactt gtggtgtcat gttggcactc agaaagtttt 240
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20

<210> 361

<211> 980

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-12156-f

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<400> 361

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 ctgatgttta ctagtatat ctcatgctag gcagttatgc tttgcttcta ggggttctc 180
 tttttaaacc aaaagaaagc tcttttcgtt ttctgtgtgc tgcattgtcc agtgtgtgtg 240
 ttacaccat cggttcttct cctctagag attagcataa ctcccttgc tgttgattg 300

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ttatatttgag caatatgttt tggaaagggtt ggttttcate atgagtgcac gcaaatcatc 360
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 caaagccaaa gaagactttt tgaaaaaatg ggagaatcca actcagaata atgccggact 480
 tgaagatttt gaaaggaaaa aaacccttgg aacaggttca tttggaagag tcatgttggg 540
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 taaactgaag caaatagagc atactttgaa tgagaaaaga atattacagg cagtgaattt 660
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 gnggcntnnc nattttcggn gtacccttcc cttttnactt aggacccctc attttttccg 900
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<210> 362

<211> 942

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-12156-r1

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<400> 362

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 tttncntttt nnnnantaag ggggggtanna nnnngggnta nttnttttc cnnnnngggg 180
 nnnnnnantn gggnccttta nnttaaaaac caaaggtecc ccaantttcn ngntttnttn 240
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 ttttangggg cccagnnttn aanngtttt natttnaaan ntttnnngn tnttnaantc 360

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caaantttna aggttggttt taaanngatt tttttttctt aaccggnnga ttttaaantt 420
 naccantnn nnnnggntng gcaanncggt gccatnnctt aaaatngann nccctttatt 480
 tttanntanc cngtttttt tnannaangg taantttant tttnnngnt nctngttna 540
 aaaatggnt tnttttttg attttaatta aaggtatttt tggtaannnt ncangacctt 600
 tttaatcng ccnnttaann gnttggaaac atttttttta ccatttccat nctngtttt 660
 ggntttagna aaaaatnttt ctccnttgg ggatttttaa ggggattttg ggtnttnc 720
 atcatnttta aatntggacc ntntanccc tntttggnnn gnttccctt tttttnaaa 780
 aattttttnn aaaaaaanaa aanacnacct gncctttgng ctgggggtaa aantttttt 840
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<210> 363

<211> 727

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-12204-f

<400> 363

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 agatataatc attttttaaa aagtgattgc ccaatgtatt tctctaacaa ttgtcacaag 180
 agaaagcata acaataaaaa tacaaaaaca tacagattta gatgtaaaat ctatataagc 240
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tgccctcagg tgaagtggat tgaaaagaca tcaaggatca aggataatca ctttgaatct 540
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 tcaaaccaca caaatatgca gatactttcc cagaatttcg cagttaaag gctgacctc 660
 ttgaaaacta accttaatgg aattctaaac atttcagttt agaatgactt tgaaaaattc 720
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<210> 364

<211> 709

<212> DNA

<213> Homo sapiens

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<223> nbla-12204-r1

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<400> 364

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 taanaaagtc ctgnnttaaca gcntntntta ncanaaaang nnggnntttt aaaacgggct 180
 tgaacttttn cncaatntgt ttggaacnct ggcntgccc cctgngtnca tntntnctgn 240
 tngngnctnt ggttttaatt ttttaattca aaaaattttg ataaaactaa aangttaaat 300
 gncecctnta atgaaatenc tgnnttaact aacncenncc ananggnttg ntttcttgg 360
 aaaatttttt aaagcntttt gaattaaagt aanaactttg ttttttaaan aacncntttt 420
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 caggaagtt tccaagctcc cctnntttac ggcaaanggg ttttgaaact ngcntttaaa 660
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<210> 365

<211> 724

<212> DNA

<213> Homo sapiens

<220>

<223> nbla-12217-f

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<400> 365

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 cacaaaccaa cagttttatg gagcgcttc tacacgtca gagataggga tacgtaagat 180
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 agcaaagacg actctgaaaa tagaatgctg cataggcaag attggatgag agagagaaga 480
 tcttgagac aggacaggta actggagatc gttcctgggc aagtgtgtat ggagaagtgt 540
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 ttaagggacc cgagaataaa ctaggattct caaaccaaac atggcccaca gacagcttgg 660
 tggacaggca tgtttttctt ttgctgcact ctacgctggg cgacagtgcg aggcctcttgt 720
 ctca 724

20

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<210> 366

<211> 869

<212> DNA

<213> Homo sapiens

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<220>

<223> nbla-12217-r1

<400> 366

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cnttttnaan anggnngncc cncncnctnn nacctaacco naaatggcct nggggggccc 240
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gggcaggggg gntnnnttna ctgnanacan nagcannnga ctaagaatga cnaagacnn 360
aachacnnnc caaggettna aagggggtng taanahngaa aacnaggggc aagggggcat 420
anggggtgag gaantnaanc ttagganccg gggggacnaa aaacanngga ngontgaacn 480
ncttgacat ngtgntacnc ttangaacga ngganacccc tgnaactcac tngncacttt 540
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nggccccaan agtttccttt ncttannana anacaaaann cntttttcaa ggccccnnnn 720
tthnnncnc gnggggncct tttaangncc canaaggcna agggncatnt gcctnctat 780
cangggnggg ggggggcntt tnccataata angccaccc nagggggggg ggncanaanc 840
cacncnagg ngnggggggg gcttcnaca 869

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【図面の簡単な説明】

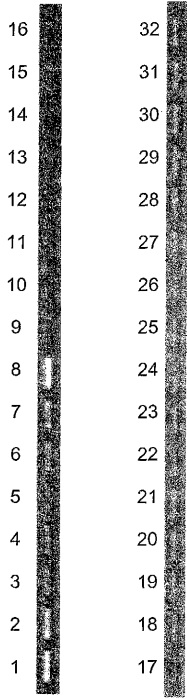
図 1 は、半定量的 P C R による予後良好・不良ヒト神経芽細胞腫における遺伝子発現量の測定結果の一例を示す電気泳動写真に対応する図である。

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図 2 は、新生マウス S C G ニューロン含む一次培養物の電子顕微鏡写真に対応する図である。

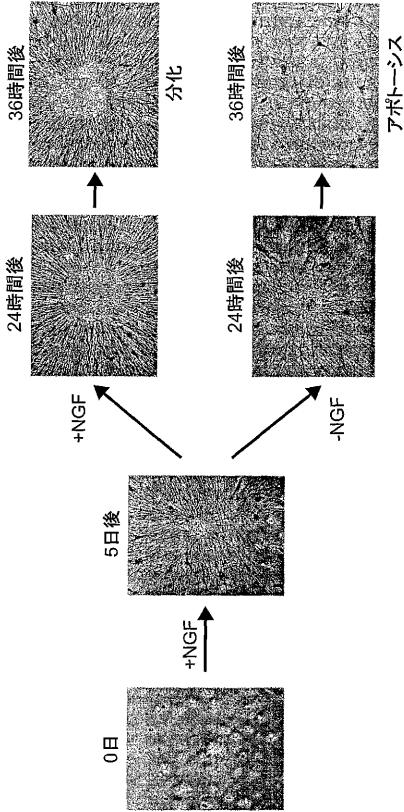
【図 1】

図 1



【図 2】

図 2



フロントページの続き

審査官 福間 信子

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国際公開第00/012139 (WO, A1)
国際公開第00/055350 (WO, A1)
NCBI, 2001年 5月 3日, Accession No.AB060967, URL, <http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?13936382:0LD09:205287>

- (58)調査した分野(Int.Cl., DB名)
C12N 15/00-90
BIOSIS/WPIDS(STN)
GenBank/EMBL/DDBJ/GeneSeq
SwissProt/PIR/GeneSeq
PubMed
JSTPlus(JDreamII)