(19)日本国特許庁(JP)　(12)公表特許公報(A)　(11)特許出願公表番号
特表2005-503753
(P2005-503753A)
(43)公表日　平成17年2月10日(2005.2.10)

| (51)Int.Cl.[7] | F I | | テーマコード（参考） |
|---|---|---|---|
| C12N 15/09 | C12N 15/00 | ZNAA | 4B024 |
| G01N 33/53 | G01N 33/53 | M | |
| G01N 33/532 | G01N 33/532 | B | |
| G01N 33/569 | G01N 33/569 | L | |

審査請求 未請求　予備審査請求 有　（全 240 頁）

(21)出願番号　特願2002-527314(P2002-527314)
(86)(22)出願日　平成13年9月6日(2001.9.6)
(85)翻訳文提出日　平成15年3月14日(2003.3.14)
(86)国際出願番号　PCT/US2001/028014
(87)国際公開番号　WO2002/022874
(87)国際公開日　平成14年3月21日(2002.3.21)
(31)優先権主張番号　60/233,177
(32)優先日　平成12年9月15日(2000.9.15)
(33)優先権主張国　米国(US)
(81)指定国　EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR),AU,CA,JP,MX,US

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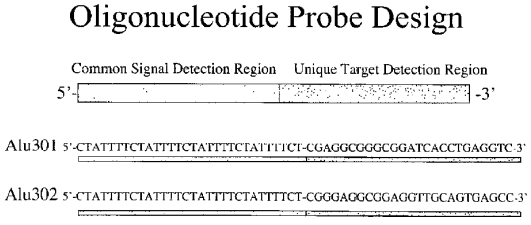
(54)【発明の名称】イン・サイチュー解析のためのオリゴヌクレオチドプローブおよびタンパク質を標識するためのオリゴヌクレオチド配列式

(57)【要約】
本発明は、細胞もしくは組織サンプル中の複数の核酸標的遺伝子または抗原を検出または局在化するためのオリゴヌクレオチドプローブおよびオリゴヌクレオチドプローブコレクションおよびタンパク質標識を提供する。具体的には、本発明は、各プローブが、配列式（ＣＴＡＴＴＴ）$_n$、（ＡＡＡＡＴＡＧ）$_n$、または（ＴＴＴＴＡＴＣ）$_n$もしくは（ＧＡＴＡＡＡＡ）$_n$［式中、すべての場合「ｎ」は１に等しいかそれを超える］をもつ標識ドメインを有する場合のイン・サイチューハイブリダイゼーション解析における使用のためのオリゴヌクレオチドプローブのコレクションを提供する。本発明は、細胞もしくは組織サンプル中の特定の核酸標的遺伝子を検出または局在化するためのオリゴヌクレオチドプローブのコレクションもしくは「カクテル」を提供する。カクテルは、次のものを検出するために有用である：κ遺伝子（配列番号：１－１６を含めて）；λ遺伝子（配列番号：５０１－５０９，５１１－５１３，および５１５）；ＣＭＶ（サイトメガロウイルス）遺伝子（配列番号：２２１－２４１を含めて）；ＥＢＥＲ（Ｅｐｓｔｅｉｎ



【特許請求の範囲】
【請求項１】
配列（ＣＴＡＴＴＴＴ）$_n$およびその補体（ＡＡＡＡＴＡＧ）$_n$［式中、「ｎ」は少なくとも１である］を含んでなるオリゴヌクレオチド標識ドメイン。
【請求項２】
レポーター分子もしくはハプテン分子により検出可能に標識された請求項１のオリゴヌクレオチド標識ドメイン。
【請求項３】
ハプテンがＯＢＥＡリンカーを介してシトシンのＮ４窒素に結合されたフルオレセインである、請求項２のオリゴヌクレオチド標識ドメイン。
【請求項４】
レポーター分子が発蛍光団である、請求項１のオリゴヌクレオチド標識ドメイン。
【請求項５】
発蛍光団が７モル％を超える密度において存在する、請求項１のオリゴヌクレオチド標識ドメイン。
【請求項６】
標識ドメインが配列ＴＣ（ＴＴＴＴＡＴＣ）$_n$（またはその相補的式）を有する、請求項１のオリゴヌクレオチド標識ドメイン。
【請求項７】
配列が配列番号：５８である、請求項１のオリゴヌクレオチド標識ドメイン。
【請求項８】
少なくとも７モル％のシトシンがＯＢＥＡリンカーによって検出可能な部分に結合されている、請求項２のオリゴヌクレオチド標識ドメイン。
【請求項９】
少なくとも２つの別個の機能性ドメイン、請求項２の標識ドメインを含んでなる第１のドメイン、および遺伝子に特異的な標的配列を含んでなる第２のドメインを有するオリゴヌクレオチドプローブ。
【請求項１０】
標識ドメインがオリゴヌクレオチドプローブの５’末端に配置され、そして遺伝子に特異的な標的配列が標識ドメインに対して３’に存在している、請求項９のオリゴヌクレオチドプローブ。
【請求項１１】
標識ドメインがオリゴヌクレオチドプローブの３’末端に配置され、そして遺伝子に特異的な標的配列が標識ドメインに対して５’に存在している、請求項９のオリゴヌクレオチドプローブ。
【請求項１２】
３つの別個の機能性ドメイン、請求項２の標識ドメインを含んでなる第１のドメイン、遺伝子に特異的な標的配列を含んでなる第２のドメイン、およびその他の標識ドメインを含んでなる第３のドメインを有し、該第２のドメインが該第１と該第３のドメインとの間に配置されているオリゴヌクレオチドプローブ。
【請求項１３】
プローブが本質的に配列番号：４０１－４１６を含めてなる群から選ばれる、κ免疫グロブリン軽鎖ｍＲＮＡまたは対応する異核（ｈｅｔｅｒｅｏｎｕｃｌｅａｒ）ＲＮＡを検出するためのプローブセット。
【請求項１４】
プローブが本質的に配列番号：５０１－５０９，５１１－５１３、および５１５からなる群から選ばれるλ免疫グロブリン軽鎖ｍＲＮＡまたは対応する異核ＲＮＡを検出するためのプローブセット。
【請求項１５】
プローブが本質的に配列番号：２２１－２４１からなる群から選ばれるサイトメガロウイ

ルス（ＣＭＶ）即時型ＲＮＡおよび／または対応するｍＲＮＡを検出するためのプローブセット。
【請求項１６】
プローブが本質的に配列番号：５１－５４からなる群から選ばれるエプスタイン・バーウイルス（ＥＢＶ）の初期ＲＮＡ、ＲＮＡ１およびＲＮＡ２、（ＥＢＥＲ）を検出するためのプローブセット。
【請求項１７】
プローブが本質的に配列番号：３０１および３０２からなる群から選ばれるヒトＡｌｕ反復サテライトゲノムＤＮＡ配列を検出するためのプローブセット。
【発明の詳細な説明】
【０００１】
発明の背景
１．発明の分野
本発明は、細胞もしくは組織サンプル中の核酸遺伝子標的を検出または局在化するためのオリゴヌクレオチドプローブおよびオリゴヌクレオチドプローブのコレクションに関する。特に、本発明はオリゴプローブのコレクションに関する。
【０００２】
２．発明の背景
イン・サイチュー解析はイン・サイチューのハイブリダイゼーションおよび免疫組織化学を含む。イン・サイチューハイブリダイゼーション（ＩＳＨ）は、細胞もしくは組織サンプル中の標的とする核酸標的遺伝子を検出または局在化するために、標的遺伝子配列もしくは転写物に対してアンチセンスである標識したＤＮＡもしくはＲＮＡプローブ分子を用いる。ＩＳＨは、発生生物学、細胞生物学および分子生物学を含む、多数の生物医療分野において有用な道具であることが分かっている。ＩＳＨは、例えば、遺伝障害を診断したり、遺伝子をマッピングしたり、遺伝子発現を研究したり、そして標的遺伝子発現の部位を局在化するために使用されてきた。
【０００３】
典型的には、ＩＳＨは、ガラススライド上に固定化した細胞もしくは組織サンプルを、細胞もしくは組織サンプル中の特定の標的遺伝子に特異的にハイブリダイズすることができる標識した核酸プローブに接触させることによって実施される（In Situ Hybridization：Medical Applications（G. R. Coulton and J. de Belleroche, eds., Kluwer Academic Publishers, 1992）；In Situ Hybridization：In Neurobiology；Advances in Methodology（J. H. Eberwine, K. L. Valentino, and J. D. Barchas, eds., Oxford University Press, 1994）；In Situ Hybridization：A Practical Approach（D. G. Wilkinson, ed., Oxford University Press, 1992））。細胞もしくは組織サンプル中の核酸への標識したプローブ分子のハイブリダイゼーションは、次に、例えば、放射能に基づく直接検出法、蛍光に基づく直接検出法、またはＢｒｄＵのようなハプテンに結合している蛍光標識したタンパク質、プローブ中に組み入れられたジゴキシゲニン標識もしくはビオチン標識したヌクレオチドの結合に基づく間接検出法を用いて検出することができる。ハプテンに基づく方法は、さらに、結合性タンパク質－酵素複合体、例えば抗体－酵素複合体によって結合されるそれらの分子および比色法に基づく検出化学を含めるように拡張された。さらに、いくつかの標的遺伝子が、複数の異なる核酸タグで標識された複数の核酸プローブに細胞もしくは組織サンプルを接触させることによって同時に解析することができる。例えば、複数の核酸プローブが、異なる発光波長をもつ複数の蛍光化合物により標識することができ、それによって単一の標的細胞もしくは組織サンプルにおいて１段階で実施される同時多重発色分析を可能にする。

【０００４】
オリゴヌクレオチドプローブ中への標識したヌクレオチドの組み入れに伴う重要な問題は、通常、ヌクレオチドに結合している複合部分が、ワトソン・クリック塩基対の形成を妨害し、その結果その標的へのプローブのハイブリダイゼーションにネガティブに影響することである。このことは、非置換シトシン（１級アミン）と形成される自然のＧ－Ｃ結合に較べて、立体障害および２級アミンの低い反応性状態への予期されるシフト（Ｎ４標識したシトシンに見られるような）のために、Ｎ４－置換されたシトシンヌクレオチドを介して結合された標識の使用に関して見られた。小さいヌクレオチド（２５～５０塩基）における何らかの小変化またはＧ－Ｃ結合の妨害は、意図した標的配列とハイブリダイズするこれらのオリゴの能力を低下させることがある。

【０００５】
オリゴヌクレオチド中に標識したヌクレオチドを組み入れるために適当なプローブ設計を開発する技術上の必要性が残っている。本発明者らは、数種の人工配列が、プローブ標識のための効果的に機能する代替物であり、そしてまた細胞もしくは組織サンプル中の核酸標的遺伝子を検出または局在化するために、単独でも、また複合したオリゴヌクレオチドプローブ混合物においても働くことを例証する。プローブコレクションのためのそのような遺伝子配列の開発および標識戦略は、医療、遺伝子および分子生物学技術における広い応用性を有する。

【０００６】
標識化学によるこの妨害およびハイブリダイゼーションストリンジェンシーおよび動力学が、本明細書において、少なくとも２つの別個の機能性ドメイン、すなわち遺伝子特異的であり、塩基対形成に必要である１つのドメインもしくは配列、およびスペーシングヌクレオチドと標識したヌクレオチドからなる人工的な非特異的配列（サンプルのゲノムに関して）である第２ドメインを有するオリゴを設計することによって解決される。これらの要素は、これらの標識ヌクレオチドがタンパク質（免疫グロブリンもしくはアビジン）を結合するためにハプテンとして一層作用しやすく、したがって遺伝子に特異的なドメインにおいてワトソン・クリック塩基対を妨害しないように置かれる。

【０００７】
発明の概要

本発明は、細胞もしくは組織サンプル中の核酸標的遺伝子を検出または局在化するために、オリゴヌクレオチドプローブ中に標識を組み入れるための新規戦略および標識されたオリゴヌクレオチドプローブのコレクションを提供する。特に、本発明は、イン・サイチューハイブリダイゼーション解析における使用のために、オリゴヌクレオチドプローブのコレクション中に組み入れることができるそのような配列の反復ポリマーを作成するための配列式を用いる非遺伝子特異的配列に関する。さらに、配列式に基づく標識された合成オリゴヌクレオチドポリマーを使用することは、結合性タンパク質、すなわち免疫グロブリンに複合された場合には、免疫組織化学的解析において使用されるそのようなタンパク質を標識するための非常に効果的な、そして制御される方法である。本発明は、細胞もしくは組織サンプル中の特定核酸標的遺伝子を検出または局在化するためのオリゴヌクレオチドプローブのコレクションもしくは「カクテル」を提供する。カクテルは、次のものを検出するために有用である：κ遺伝子（配列番号：１－１６を含めて）；λ遺伝子（配列番号：５０１－５０９，５１１－５１３，および５１５）；ＣＭＶ（サイトメガロウイルス）遺伝子（配列番号：２２１－２４１を含めて）；ＥＢＥＲ（Ｅｐｓｔｅｉｎ－Ｂａｒｒ初期ＲＮＡ）遺伝子（配列番号：５１－５４を含めて）；Ａｌｕ（配列番号：５５－５６）；ポリＡ（配列番号：５７）；および検出テール（配列番号：３３０）。

【０００８】
本発明は、配列（ＣＴＡＴＴＴＴ）$_n$およびその補体（ＡＡＡＡＴＡＧ）$_n$［式中、「ｎ」は少なくとも１である］を含んでなるオリゴヌクレオチド標識ドメインに向けられる。

【０００９】
また、本発明は、少なくとも２つの別個の機能性ドメイン、請求項２の標識ドメインを含

んでなる第１のドメイン、および遺伝子に特異的な標的配列を含んでなる第２のドメインを有するオリゴヌクレオチドプローブに向けられる。

【００１０】

また、本発明は、プローブが本質的に配列番号：４０１－４１６を含めてなる群から選ばれるκ免疫グロブリン軽鎖ｍＲＮＡまたは対応する異核（ｈｅｔｅｒｅｏｎｕｃｌｅａｒ）ＲＮＡを検出するためのプローブセットに向けられる。

【００１１】

また、本発明は、プローブが本質的に配列番号：５０１－５０９，５１１－５１３、および５１５からなる群から選ばれるλ免疫グロブリン軽鎖ｍＲＮＡまたは対応する異核ＲＮＡを検出するためのプローブセットに向けられる。

【００１２】

また、本発明は、プローブが本質的に配列番号：２２１－２４１からなる群から選ばれるサイトメガロウイルス（ＣＭＶ）即時型ＲＮＡおよび／または対応するｍＲＮＡを検出するためのプローブセットに向けられる。

【００１３】

また、本発明は、プローブが本質的に配列番号：５１～５４からなる群から選ばれるＥｐｓｔｅｉｎ－Ｂａｒｒウイルス（ＥＢＶ）初期ＲＮＡ、ＲＮＡ１およびＲＮＡ２、（ＥＢＥＲ）を検出するためのプローブセットに向けられる。

【００１４】

また、本発明は、プローブが本質的に配列番号：３０１および３０２からなる群から選ばれるヒトＡｌｕ反復サテライトゲノムＤＮＡ配列を検出するためのプローブセットに向けられる。

【００１５】

本発明の特定の好適な実施態様は、以下のある好適な実施態様および請求項のより詳細な記述から明らかになるであろう。

【００１６】

図１は、２ドメインプローブ設計の遺伝子プローブ構造を図解している。これは、次の実施例において記述される遺伝子特異的カクテルにおけるプローブのために使用されるオリゴヌクレオチドの設計である。各プローブは、２つのドメイン：５’標識ドメインおよび３’標的遺伝子の標的遺伝子に特異的なドメインからなる。標識ドメインは、この特異配列（ＣＴＡＴＴＴＴ）$_n$からなり、この場合、各シトシンは、発蛍光団もしくはシトシン－ハプテン複合体により標識されてもよく、この実施態様ではハプテンはフルオレセインである。この図は、具体的には、３０１（配列番号：５５）および３０２（配列番号：５６）プローブについての核酸配列を示し、これらの各々は、ヒト反復Ａｌｕ配列に対応する標的遺伝子の遺伝子特異的ドメインおよびフルオレセインハプテンを有する標識ドメインを保持する。

【００１７】

図２は、標識ドメインを含んでなるプローブ（３３０プローブ；配列番号：５８）を用いるヒト皮膚組織のイン・サイチューハイブリダイゼーション（ＩＳＨ）解析について得られた結果を説明する。検出可能なシグナルの不在は、これらのＩＳＨ実施例において使用されたオリゴヌクレオチドに共通する標識ドメインの配列式（ＣＴＡＴＴＴＴ）$_n$が、それがハイブリダイズしないためにヒト核酸配列とワトソン・クリック塩基対を形成する能力において非特異的で、そして非反応性であることを示している。

【００１８】

図３は、標識ドメインおよびポリｄ（Ｔ）標的遺伝子特異的ドメインを含んでなるプローブ（３２０プローブ；配列番号：５７）を用いるヒト皮膚組織のＩＳＨ解析について得られた結果を説明する。細胞質に局在される検出可能なシグナルの存在は、このプローブがメッセンジャーＲＮＡのポリアデニル化領域に特異的にハイブリダイズすることができることを示している。

【００１９】

図４Ａ－４Ｂは、組織サンプルが、イン・サイチューハイブリダイゼーション前にリボヌクレアーゼＡにより処理されなかった（Ａ）か、またはイン・サイチューハイブリダイゼーション前にリボヌクレアーゼＡにより処理された（Ｂ）場合の、３２０プローブを用いるヒト皮膚組織のＩＳＨ解析について得られた結果を説明する。（Ｂ）における検出可能なシグナルにおける減少は、このプローブがメッセンジャーＲＮＡに共通するポリアデニル化領域に特異的にハイブリダイズすることを示している。

【００２０】

図５Ａ－５Ｂは、ハイブリダイゼーションおよびストリンジェンシー洗浄が室温（Ａ）または３７℃（Ｂ）で実施された場合の、３２０プローブを用いるヒト脾臓組織のＩＳＨ解析について得られた結果を説明する。この結果は、より強い発色がより低いストリンジェント条件を示すことによって、発色の強度がハイブリダイゼーション条件のストリンジェンシーに関係することを示している。

【００２１】

図６は、３２０プローブを用いるヒトＲａｊｉ細胞系のＩＳＨ解析について得られた結果を説明する。これは、このプローブ設計がまた包埋細胞系ならびに包埋組織を用いて機能することを示している。

【００２２】

図７は、３０１および３０２プローブからなるプローブコレクションを用いるヒトＲａｊｉ細胞系のＩＳＨ解析について得られた結果を説明する。

【００２３】

図８は、３０１および３０２プローブからなるプローブコレクションを用いるヒトＨＴ細胞系のＩＳＨ解析について得られた結果を説明する。

【００２４】

図９は、３０１および３０２プローブからなるプローブコレクションを用いるラット細胞系のＩＳＨ解析について得られた結果を説明する。検出可能なシグナルの不在は、このプローブコレクションがヒト核酸配列に特異的であることを示している。

【００２５】

図１０は、エプスタイン・バーウイルス（ＥＢＶ）　ＥＢＥＲ核ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブ［配列番号：５１～配列番号：５４？］を用いるＥＢＶ陰性ヒトＨＴ細胞系のＩＳＨ解析について得られた結果を説明する。

【００２６】

図１１は、ＥＢＶ　ＥＢＥＲ１および２核ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：５１～配列番号：５４］を用いるヒト脾臓組織のＩＳＨ解析について得られた結果を説明する。

【００２７】

図１２は、ＥＢＶ　ＥＢＥＲ１および２核ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：５１～配列番号：５４］を用いるヒト扁桃組織のＩＳＨ解析について得られた結果を説明する。

【００２８】

図１３Ａ－１３Ｂは、ＥＢＶ　ＥＢＥＲ１および２核ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：５１～配列番号：５４］を用いるヒト脾臓組織のＩＳＨ解析について得られた結果を説明するが、この場合、組織サンプルは、イン・サイチューハイブリダイゼーション前にリボヌクレアーゼＡにより処理されなかった（Ａ）か、またはイン・サイチューハイブリダイゼーション前にリボヌクレアーゼＡにより処理された（Ｂ）。（Ｂ）における検出可能なシグナルにおける減少は、このプローブがヒトＥＢＥＲ１および２核ＲＮＡに特異的にハイブリダイズすることを示している。

【００２９】

図１４は、ヒト免疫グロブリンλ軽鎖ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブ［配列番号：１５］を用いるκ軽鎖ポジティブヒト扁桃組織のＩＳＨ解析

について得られた結果を説明する。

【００３０】

図１５は、ヒト免疫グロブリンλ軽鎖ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：２－４、配列番号：７－１２、配列番号：１４，１５］を用いるリンパ腫組織のＩＳＨ解析について得られた結果を説明する。（Ａ）のリンパ腫組織はκ軽鎖を過発現し、そして（Ｂ）の組織はλ軽鎖を過発現する。（Ｂ）における検出可能なシグナルの不在は、κ軽鎖プローブコレクションがκ軽鎖ｍＲＮＡに特異的であることを示している。

【００３１】

図１６は、ヒト免疫グロブリンλ軽鎖可変部ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブ［配列番号：１９－２９］を用いるλ軽鎖ポジティブヒト扁桃組織のＩＳＨ解析について得られた結果を説明する。

【００３２】

図１７は、ヒト免疫グロブリンλ軽鎖ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：１９－２９］を用いるλ軽鎖ポジティブヒトＲＰＭＩ　８２２６細胞系のＩＳＨ解析について得られた結果を説明する。

【００３３】

図１８Ａ－１８Ｂは、ヒト免疫グロブリンλ軽鎖ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：１９－２９］を用いるヒト脾臓組織のＩＳＨ解析について得られた結果を説明する。（Ａ）の組織はλ軽鎖を過発現し、そして（Ｂ）の組織はκ軽鎖を過発現する。（Ｂ）における検出可能なシグナルの不在は、λ軽鎖プローブコレクションがヒトλ軽鎖ｍＲＮＡに特異的であることを示している。

【００３４】

図１９は、ＣＭＶ即時型ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：３０－３２，配列番号：３４－３５，配列番号：３８，配列番号：５０］を用いるサイトメガロウイルス（ＣＭＶ）ポジティブヒト肺組織のＩＳＨ解析について得られた結果を説明する。［ＣＭＶ感染細胞］

図２０は、ＣＭＶ即時型ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：３０－３２，配列番号：３４－３５，配列番号：３８，配列番号：５０］を用いる、ＣＭＶ即時型ＲＮＡの発現がシクロヘキサミドによって誘発されなかったラット９Ｇ細胞系のＩＳＨ解析について得られた結果を説明する。

【００３５】

図２１Ａ－２１Ｂは、ＣＭＶ即時型ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：３０－３２，配列番号：３４－３５，配列番号：３８，配列番号：５０］を用いる、ＣＭＶ即時型ＲＮＡの発現がシクロヘキサミドによって誘発されたラット９Ｇ細胞系のＩＳＨ解析について得られた結果を説明する。（Ａ）の組織は倍率４０Ｘにおいて示され、そして（Ｂ）の組織は倍率２０Ｘにおいて示される。

【００３６】

好適な実施態様の詳細な記述

本発明は、細胞もしくは組織サンプル中の核酸標的遺伝子を検出または局在化するためのオリゴヌクレオチドプローブおよびオリゴヌクレオチドプローブコレクションを提供する。特に、本発明は、イン・サイチューハイブリダイゼーション解析における使用のためのオリゴヌクレオチドプローブのコレクションに関する。

【００３７】

より具体的には、本発明は、オリゴヌクレオチドプローブまたはタンパク質に検出可能な部分（標識）を結合するためのヌクレオチドポリマーまたは標識ドメインについての特定

の配列式の使用に関する。これらの配列もしくはその誘導体の特定の利用は、ハイブリダイゼーション条件の標準ストリンジェンシー下では検出可能なレベルにおいてヒトＤＮＡもしくはＲＮＡにハイブリダイズしないという不活性または非反応性の特性である。これらの標識ドメインまたはポリマーは、イン・サイチューハイブリダイゼーション解析において細胞もしくは組織サンプル中の遺伝子に特異的な配列を検出するためのオリゴヌクレオチドプローブ中への組み入れに有用な遺伝子配列であることが例証された。さらに、この不活性な配列のセットは、免疫組織化学的解析においてハプテンおよび抗原を検出するために、免疫グロブリンもしくは他のタンパク質に標識を結合させるために有用である。

【００３８】

本明細書で使用されるように、用語「プローブ」もしくは「オリゴヌクレオチドプローブ」は、相補的核酸標的遺伝子を検出するために使用される核酸分子を指す。

【００３９】

本明細書で使用されるように、用語「ハイブリダイゼーション」は、相補的核酸配列が接合して二本鎖核酸分子を形成する過程を指す。例えば、放射性もしくは蛍光タグにより標的核酸分子を標識することによって、プローブと標的遺伝子間の相互作用が検出できる。

【００４０】

本発明のオリゴヌクレオチドプローブおよびコレクションのオリゴヌクレオチドプローブは慣用の方法を用いて合成される。参照、例えば、Ｍｅｔｈｏｄｓ ｉｎ Ｍｏｌｅｃｕｌａｒ Ｂｉｏｌｏｇｙ，Ｖｏｌ２０：Ｐｒｏｔｏｃｏｌｓ ｆｏｒ Ｏｌｉｇｏｎｕｃｌｅｏｔｉｄｅｓ ａｎｄ Ａｎａｌｏｇｓ １６５－８９（Ｓ．Ａｇｒａｗａｌ，ｅｄ．，１９９３）；Ｏｌｉｇｏｎｕｃｌｅｏｔｉｄｅｓ ａｎｄ Ａｎａｌｏｇｕｅｓ：Ａ Ｐｒａｔｉｃａｌ Ａｐｒｏａｃｈ ８７－１０８（Ｆ．Ｅｃｋｓｔｅｉｎ，ｅｄ．，１９９１）。

【００４１】

本発明の好適な実施態様では、オリゴヌクレオチドプローブは、２つの別個のドメイン：５’（または標識）ドメインおよび３’（または遺伝子に特異的な標的）ドメインを保持する（参照、図１Ａ）。本発明のより好適な実施態様では、オリゴヌクレオチドプローブは、配列（ＣＴＡＴＴＴＴ）$_n$からなる標識ドメインを保持する。また、他の実施態様が本明細書において例証され、これは２つの末端の標識ドメインおよび中央の遺伝子特異的標的ドメインを有する３重ドメイン態様も含む。具体的には、配列番号：１２５－１２６がこの標識スキームを表している。なお、標識ドメインのさらなる好適な実施態様は、ＴＣ（ＴＴＴＴＡＴＣ）$_n$またはその補体である。この配列は（ＣＴＡＴＴＴＴ）$_n$標識ドメインと同じくらい独特であると予想される。本発明のオリゴヌクレオチドプローブは、特定の細胞もしくは組織において該プローブと標的核酸との間のハイブリダイゼーションが検出できるように標識される。イン・サイチューハイブリダイゼーション（ＩＳＨ）解析における使用のために許容される標識は当業者には既知である。そのような標識は、例えば、放射能に基づく直接検出法、蛍光に基づく直接検出法、蛍光に基づく直接検出法と共役されたジゴキシゲニン標識もしくはビオチン標識したプローブ、または抗体－酵素に基づく検出法と共役されたジゴキシゲニン標識もしくはビオチン標識したプローブを用いて、プローブと標的遺伝子間の相互作用が検出されるのを可能にする。本発明の好適な実施態様では、オリゴヌクレオチドプローブはフルオレセインにより標識される。本発明のより好適な実施態様では、オリゴヌクレオチドプローブは、シトシンヌクレオチドが、直接検出のための発蛍光団、または間接検出のためのハプテンにより標識されてもよい配列（ＣＴＡＴＴＴＴ）$_n$からなる標識ドメインを保持する。いずれにおいても、フルオレセイン－シトシンヌクレオチド複合体およびフルオレセイン分子は、ＯＢＥＡ結合を介してシトシンのＮ４位に結合される（引用によって本明細書に組み入れられているＭｉｓｈｒａ ｅｔ ａｌ．，米国特許第５，６８４，１４２号参照）。好適な実施態様では、標識ドメインに結合される発蛍光団の密度は、標識ドメインのみに対して測定された場合、少なくとも７モル％、好ましくは少なくとも１０モル％、もっとも好ましくは少なくとも１６モル％である。例えば、プローブ４０１が考えられる場合（２ドメインプローブ）、そ

れは、Ｃがまた標識される３’末端ＣＴを含む３０塩基の標識ドメインを含有し、モル％は５／３０＝１６．７モル％標識である。プローブ全体では、モル％は８．３である。

【００４２】

本発明の若干の実施態様では、いくつかの標的遺伝子が、複数の異なる核酸タグで標識された複数の核酸プローブに細胞もしくは組織サンプルを接触させることによって同時に解析される。例えば、複数の核酸プローブが、異なる発光波長をもつ複数の蛍光化合物により標識でき、それによって単一の標的細胞もしくは組織サンプルにおいて１段階で実施される同時多色分析を可能にする。

【００４３】

本発明のオリゴヌクレオチドプローブおよびオリゴヌクレオチドプローブコレクションは、細胞もしくは組織サンプル中の核酸標的遺伝子を検出または局在化するためにＩＳＨ解析において使用することができる。ＩＳＨ解析は、例えば、In Situ Hybridization：Medical Applications（G. R. Coulton and J. de Belleroche, eds., Kluwer Academic Publishers, １９９２）；In Situ Hybridization：In Neurobiology；Advances in Methodology（J. H. Eberwine, K. L. Valentino, and J. D. Barchas, eds., Oxford University Press, １９９４）；またはIn Situ Hybridization：A Practical Approach（D. G. Wilkinson, ed., Oxford University Press, １９９２）において記述されるように実施されてもよい。

【００４４】

本発明のプローブおよびプローブコレクションの好適な実施態様は、図１－２１および実施例１－２を参照することによってもっとも良く理解される。以下に示す実施例は、本発明の特別な実施態様および種々のその使用の具体的説明である。それらは、説明の目的のためにのみ記述され、そして本発明を限定するものとして解釈されるべきではない。

【００４５】

実施例１

プローブコレクションの調製

長さ５５～６０塩基の多数のオリゴヌクレオチドプローブからなるプローブコレクションが次のように設計された。この実施例では、各オリゴヌクレオチドプローブは、２つの別個のドメイン：５’（または標識）ドメインおよび３’（または標的遺伝子に特異的）ドメインを保持した（参照、図１）。

【００４６】

この実施態様では、標識ドメインは配列$(CTATTTT)_n$からなり、この場合、シトシンヌクレオチドは、フルオレセイン－シトシンヌクレオチド複合体を表し、そしてフルオレセイン分子は、ＯＢＥＡ結合を介してシトシンのＮ４位に結合される。

【００４７】

標的遺伝子に特異的なドメインは、特定核酸標的遺伝子に対して相補的である２５－３０塩基配列からなる。オリゴヌクレオチドプローブは、ヒト免疫グロブリンκ軽鎖可変部（参照、表１；オリゴヌクレオチドプローブ４０１－４１６）、ヒト免疫グロブリンλ軽鎖可変部（オリゴヌクレオチドプローブ５０１－５１５）、ヒトサイトメガロウイルス（ＣＭＶ）配列（オリゴヌクレオチドプローブ２２１－２４１）、ヒトＥｐｓｔｅｉｎ－Ｂａｒｒウイルス（ＥＢＶ）ＥＢＥＲ（Ｅｐｓｔｅｉｎ－Ｂａｒｒ初期ＲＮＡ）配列（オリゴヌクレオチドプローブ１００Ａ２，１００Ｃ２，１００Ａ１および１００Ｂ１），ヒト反復Ａｌｕ配列（オリゴヌクレオチドプローブ３０１および３０２）、およびポリｄ（Ｔ）（オリゴヌクレオチドプローブ３２０）に対応する標的遺伝子特異的ドメインを保持するよう設計された。

【００４８】

実施例２

標識ドメイン設計：Ａｌｕ反復配列プローブ

Ａｌｕヒト反復配列に対するすべて４プローブが使用されて標識ドメイン設計を評価した。プローブ番号３０１（配列番号：５５）、３０１Ａ（配列番号：１１６）、３０１Ａ２／２（配列番号：１２１）、および３０１Ａ３／２（配列番号：１２２）が表１に示される。

【００４９】

４プローブは、それぞれ、反応液中プローブ１ｍｌ当たり濃度１００，７５，５０および２５ｎｇ／ｍｌにおいて評価された。このハイブリダイゼーション解析は、標準プロトコールを用いてマニュアルで実施された。標的、パラフィン包埋細胞系ＭＢＡ ＭＤ４６８（Ｏｎｃｏｒ ＩＮＦＯＲＭ™ Ｈｅｒ－２／ｎｅｕ Ｃｏｎｔｒｏｌ Ｓｌｉｄｅｓ，Ｃａｔ．Ｎｏ．Ｓ８１００，Ｌｅｖｅｌ １，Ｖｅｎｔａｎａ Ｍｅｄｉｃａｌ Ｓｙｓｔｅｍｓ，Ｉｎｃ．，Ｔｕｃｓｏｎ，ＡＺ）が標的サンプルであり、そして標準キシレン法によってパラフィンを除去することによって処理された。組織は、ＶｅｎｔａｎａのＡＰＫバッファーによる１：２希釈液として、５０℃で１２分間ＶｅｎｔａｎａのＰｒｏｔｅａｓｅ １にさらされた。ハイブリダイゼーション反応は、２ＸＳＳＣ／ＴｒｉｔｏｎＸ－１００の残留１００ｕｌ容量に対して、１００ｕｌプローブとしてのプローブ希釈液（２５％ホルムアミド、５％デキストラン硫酸、２ＸＳＳＣ、１％Ｔｒｉｔｏｎ）の添加により達成された。スライドは８５℃で５分間加熱され、次いで、３７℃で１時間インキュベートされた。標準ＳＳＣ洗浄が過剰のプローブを除去するために続いた。ハイブリッドはＦＩＴＣに対する抗体により検出された。マウス抗体は、Ｖｅｎｔａｎａ Ｅｎｈａｎｃｅｄ Ａｌｋａｌｉｎｅ Ｐｈｏｓｐｈａｔａｓｅ Ｂｌｕｅ Ｄｅｔｅｃｔｉｏｎ（ｃａｔ＃７６０－０６１）を用いて比色法により検出された。特に指摘しなければ、すべての試薬はＶｅｎｔａｎａ Ｍｅｄｉｃａｌ Ｓｙｓｔｅｍｓ，Ｉｎｃ．，Ｔｕｃｓｏｎ，ＡＺから得られた。結果は明視野顕微鏡を用いて発色検出によって観察された。

【００５０】

これらの実験の結果は、シグナル強度がプローブに共役されたフルオレセインハプテンの総数の関数であり、そしてシグナルが特定の標識ドメイン設計に属することであった。プローブ分子当たりのフルオレセイン数が大きいほど、より大きいシグナルが観察された。設計の比較およびプローブへのハプテンの配置は、これがシグナルの強度におけるファクターではないことを示した。５フルオレセインを含有した２つのプローブ（３０１Ａ３／２（配列番号：１２２）および３０１（配列番号：５５））両方は等しいシグナルを生じた。これらの２つのプローブは、３０１Ａ２／２、４フルオレセインを有する分離した標識ドメイン設計を有するプローブ、について見られるよりも大きいシグナルを生じた。プローブ３０１Ａ２／２は、プローブ３０１Ａ、５’末端に１つの標識ドメイン設計を有し、そして３フルオレセインを有するプローブ、よりも大きいシグナルを生じた。

【００５１】

実施例３

標識ドメイン設計：ＥＢＥＲプローブ

この実験は、フルオレセインハプテン間のより大きい間隔が、イン・サイチューハイブリダイゼーション解析におけるプローブ検出段階におけるシグナル生成を改善できるか否かを決定するために、２つの標識ドメイン設計および配列を比較した。

【００５２】

使用された組織は、シラン＋ガラス顕微鏡スライド上に置かれた厚さ４ミクロンの中性バッファーホルマリンパラフィン包埋切片中に固定されたＥＢＶ感染ヒト脾臓組織であった。組織切片は、Ｖｅｎｔａｎａ ＤＩＳＣＯＶＥＲＹ™ 機器において脱パラフィンされ、続いて温度３７℃でＶｅｎｔａｎａのＰｒｏｔｅａｓｅ１により６分間消化された。プローブは、Ｖｅｎｔａｎａ Ｍｅｄｉｃａｌ Ｓｙｓｔｅｍｓ，Ｉｎｃ．の自動ＩＳＨ染色システム，Ｄｉｓｃｏｖｅｒｙによって調製された後にスライド上に残された等容量の２ＸＳＳＣ／ＴｒｉｔｏｎＸ－１００残留容量に対して適用される１００ｕｌとして、濃度５０ｎｇ／ｍｌにおいてハイブリダイゼーションバッファー希釈液に溶解された。プロ

ーブ希釈液は３７℃で６分間スライド上で残留容量と混合され、次いで、溶液は８５℃に加熱され、そして全１０分間そこで維持された。次いで、スライドは温度３７℃にされ、そしてその温度で１時間維持された。スライド上のこれらの水性反応のすべては、処理中の水の蒸発損失を防ぐためにＬＩＱＵＩＤ ＣＯＶＥＲＳＬＩＰ$^{TM}$のフィルム下ですべて実施された。ハイブリダイゼーション後の各スライドは、各洗浄間に６分間インキュベーションしつつ２ＸＳＳＣ／Ｔｒｉｔｏｎ溶液で３回洗浄され、スライド容量は約３００ｕｌ（＋／－１０％ｖｏｌ）であった。ハイブリッドはＦＩＴＣに対する抗体により検出された。マウス抗体は、Ｖｅｎｔａｎａ Ｅｎｈａｎｃｅｄ Ａｌｋａｌｉｎｅ Ｐｈｏｓｐｈａｔａｓｅ Ｂｌｕｅ Ｄｅｔｅｃｔｉｏｎ（ｃａｔ＃７６０－０６１）を用いて比色法により検出された。

【００５３】

この研究のために使用された２つのオリゴヌクレオチドプローブは、プローブ１００Ａ１（配列番号：５３）および１００２Ａ３２（配列番号：１２０）である。これらのプローブの間の２つの差異は標識ドメイン配列と構造であった。配列式（ＣＴＡＴＴＴＴ）$_4$ＣＴ（配列番号：５８）を有するプローブ１００Ａ１の標識ドメインは、遺伝子標的ドメインに対して５’に存在し、ＯＢＥＡリンカーを介してシトシン残基に結合された５フルオレセインを含有していた。オリゴプローブ１００２Ａ３２の標識ドメインは（配列番号：１２５）に類似していた。異なる配列以外に、主要な差異は、シトシン間隔が７塩基離れて比較的近くに存在するオリゴ１００Ａ１に較べて、フルオレセイン標識されたシトシンが、１０塩基離れて間隔を置かれているということである。Ｈスコア解析によって推定されるこの比較の結果は、これらのオリゴヌクレオチドがスライド上で生成されたシグナル量に関して同等であることであった。データは、１００Ａ２については、３視野のすべてにおいて解析された３６８細胞ではＨスコアは１０６であり、そしてプローブ１００２Ａ３２については、３視野において解析された３４５細胞ではＨスコアは１０９であることであった。Ｈスコアは、スライド上の総標的特異的シグナルの相対的比較を得るために、組織切片におけるスコアバックグラウンド対シグナル比に因数分解する、顕微鏡を用いて実施される分光写真解析である。（参照、引用文献Ｇｉｒｏｕｄ，Ｆ．Ｐｅｒｒｉｎ Ｃ，ａｎｄ Ｓｉｍｏｎｙ Ｌａｆｏｎｔａｉｎｅ，Ｊ．；Ｑｕａｎｔｉｔａｔｉｖｅ Ｉｍｍｕｎｏｃｙｔｏｃｈｅｍｉｓｔｒｙ ａｎｄ Ｉｍｍｕｎｏｈｉｓｔｏｃｈｅｍｉｓｔｒｙ．Ｔｈｉｒｄ Ｃｏｎｆｅｒｅｎｃｅ ｏｆ ｔｈｅ Ｅｕｒｏｐｅａｎ Ｓｏｃｉｅｔｙ ｆｏｒ Ａｎａｌｙｔｉｃａｌ Ｃｅｌｌｕｌａｒ Ｐａｔｈｏｌｏｇｙ，１９９４；ａｎｄ ＡｕｔｏＣｙｔｅ Ｑｕｉｃ Ｉｍｍｕｎｏ Ｕｓｅｒ’ｓ Ｍａｎｕａｌ，１９９８，ｄｏｃｕｍｅｎｔ ｎｕｍｂｅｒ ＰＡ－０２９，Ｃｏ ＡｕｔｏＣｙｔｅ Ｉｎｃ．Ｂｕｒｌｉｎｇｔｏｎ ＮＣ２７２１）。ヒストグラムおよびスコアシートは、各オリゴが比色シグナルを生じる上で等しく有効であったことを示している。このことは、標識ドメインの位置が遺伝子標的配列に対して３プライムであっても５プライムであってもよいか、または遺伝子標的配列が２つの標識ドメイン間に位置していてもよいことを示している。

【００５４】

実施例４

イン・サイチューハイブリダイゼーション

実施例１において調製されたプローブコレクションは、最初に、２０％デキストラン硫酸（ｗｔ／ｖｏｌ）、５０％ホルムアミド（ｖｏｌ／ｖｏｌ）、２ＸＳＳＣ、１０ｍＭｔｒｉｓ－ＨＣｌ、５ｍＭＥＤＴＡおよび０．０５％Ｂｒｉｊ－３５からなる溶液において最終ｐＨ７．３において希釈された。次いで、プローブコレクションは２ＸＳＳＣおよび０．０５％ＴｒｉｔｏｎＸ－１００からなる溶液の等容量と混合された。

【００５５】

ＩＳＨ解析のためのサンプルは、ホルマリン固定され、パラフィン包埋された細胞もしくは組織サンプルを４μｍの切片に切断し、そして切片をガラススライド上に置くことによって調製された。続くサンプルの処理およびＩＳＨは、自動装置、例えば、両方とも引用

によって本明細書に組み入れられている同時所有で同時係属中の米国特許出願第６０／０７６，１９８号および同第０９／２５９，２４０号に記述されているＤＩＳＣＯＶＥＲＹ™ Ａｕｔｏｍａｔｅｄ ＩＳＨ／ＩＨＣ Ｓｔａｉｎｅｒ（Ｖｅｎｔａｎａ Ｍｅｄｉｃａｌ Ｓｙｓｔｅｍｓ，Ｉｎｃ．Ｔｕｃｓｏｎ，ＡＺ）において実施された。サンプルからパラフィンを除去するために、スライドは水溶液に浸漬され、約２０分間加熱され、次いで洗浄された。自動脱パラフィン操作は、両方とも引用によって本明細書に組み入れられている米国特許出願第６０／０９９、０１８号および同第０９／２５９，２４０号に一層完全に記述されている。次いで、サンプルはプロテアーゼで処理され、そしてスライドは、８５℃（ＲＮＡ標的遺伝子に対するハイブリダイゼーションのため）もしくは９０－９５℃（ＤＮＡ標的遺伝子に対するハイブリダイゼーションのため）に４～１０分間加熱された。

【００５６】

ハイブリダイゼーション反応液は、典型的には、１０％デキストラン硫酸（ｗｔ／ｖｏｌ）、２５％ホルムアミド（ｖｏｌ／ｖｏｌ）、２ＸＳＳＣ、５ｍＭｔｒｉｓ、２．５ｍＭＥＤＴＡ，０．０２５％Ｂｒｉｊ－３５，ＴｒｉｔｏｎＸ－１００、および各個々のプローブ分子２５～１２５ｎｇ／ｍｌからなるハイブリダイゼーションバッファーにおいて行われた。ＩＳＨ反応は３７℃～５４℃において実施された。実施例１において記述されるプローブコレクションを用いるＩＳＨでは、ハイブリダイゼーション反応は場合によっては４７℃で１時間実施された（ハイブリダイゼーション反応が場合によっては３７℃で１時間実施されたポリｄ（Ｔ）プローブを除く）。

【００５７】

サンプル中の特定の標的遺伝子に対するフルオレセイン標識プローブ分子のハイブリダイゼーションは、一連の結合性タンパク質を用いることによって検出された、すなわち二次抗体検出。しかしながら、結合されたプローブを可視化できるｄｅｔｅｃｔ検出法を使用することも同じく可能である。二次検出では、最初に、フルオレセイン標識されたプローブ分子に対向された抗フルオレセインマウスモノクローナル抗体がサンプルに添加された。次に、マウス抗体に対向したビオチン標識ポリクローナルヤギ抗体がサンプルに添加された。最後に、ハイブリダイゼーション反応物は、リン酸５－ブロモ－４－クロロ－３－インドリル／ニトロブルーテトラゾリウム（ＢＣＩＰ／ＮＢＴ）基質を用いて比色法により検出された。この技術は、「二次抗体検出」と呼ばれ、当業者には日常的に行われる。一次および二次抗体は、Ｖｅｎｔａｎａ Ｍｅｄｉｃａｌ Ｓｙｓｔｅｍｓ，Ｉｎｃ．Ｔｕｃｓｏｎ，ＡＺを含む多くの販売元から得ることができ、これらはＶｅｎｔａｎａ自動染色システム（ＥＳ®，ＮｅｘＥＳ®，ＤＩＳＣＯＶＥＲＹ™，およびＢＥＮＣＨＭＡＲＫ™）において使用するために最適化されている。

【００５８】

図２－２１は、図１において図解される構造モチーフを有する本明細書で開示され、特許請求されるプローブまたはそのようなプローブからなるプローブコレクションを使用して、種々の細胞系もしくは組織サンプルのイン・サイチューハイブリダイゼーション解析について得られた結果を説明している。

【００５９】

【表１】

表1

| プローブ ID | 配列 | 配列 ID |
|---|---|---|
| 401 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCAGAGTAGCAGGAGCCCCAGGAGCTGAGC-3' | 1 |
| 402 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGATGGAGACTGGGTCAACTGGATGTCACA-3' | 2 |
| 403 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GCAAGCGATGGTGACTCTGTCTCCTACAGC-3' | 3 |
| 404 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TCTGTCCCAGATCCACTGCCACTGAACCTT-3' | 4 |
| 405 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GCAGCCACAGTTCGCTTCATCTGCACCTTG-3' | 5 |
| 406 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TTTCAACTGCTCATCAGATGGCGGGAAGAT-3' | 6 |
| 407 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AAGTTATTCAGCAGGCACACAACAGAGGCA-3' | 7 |
| 408 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGCGTTATCCACCTTCCACTGTACTTTGGC-3' | 8 |
| 409 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TAGGTGCTGTCCTTGCTGTCCTGCTCTGTG-3' | 9 |
| 410 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GTAGTCTGCTTTGCTCAGCGTCAGGGTGCT-3' | 10 |
| 411 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GATGGGTGACTTCGCAGGCGTAGACTTTGT-3' | 11 |
| 412 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CTCTCCCCTGTTGAAGCTCTTTGTGACGGG-3' | 12 |
| 413 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TGGAACTGAGGAGCAGGTGGGGGCACTTCT-3' | 13 |
| 414 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GAAAAAGGGTCAGAGGCCAAAGGATGGGAG-3' | 14 |
| 415 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AGATGAGCTGGAGGACCGCAATAGGGGTAG-3' | 15 |

【００６０】
【表２】

| | | |
|---|---|---|
| 416 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GCATAATTAAAGCCAAGGAGGAGGAGGGGG-3' | 16 |
| 501 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCTGAGTGAGGAGGGTGAGGAGCAGCAGAG-3' | 17 |
| 502 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AGACCCAGACACGGAGGCAGGCTGAGTCAG-3' | 18 |
| 503 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TGTTGGTTCCAGTGCAGGAGATGGTGATCG-3' | 19 |
| 504 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TAAATCATGATTTTGGGGGCTTTGCCTGGG-3' | 20 |
| 505 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TGTTGCCAGACTTGGAGCCAGAGAAGCGAT-3' | 21 |
| 506 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AATAATCAGCCTCGTCCTCAGCCTGGAGCC-3' | 22 |
| 507 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGTCCCTCCGCCGAAAACCACAGTGTAACT-3' | 23 |
| 508 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TTATGAGACACACCAGTGTGGCCTTGTTGG-3' | 24 |
| 509 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CTGCTCAGGCGTCAGGCTCAGATAGCTGCT-3' | 25 |
| 511 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT ATGCGTGACCTGGCAGCTGTAGCTTCTGTG-3' | 26 |
| 512 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT ATTCTGTAGGGGCCACTGTCTTCTCCACGG-3' | 27 |
| 513 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCTCCCCTGGGATCCTGCAGCTCTAGTCTC-3' | 28 |
| 515 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TGAGGGTTTATTGAGTGCAGGGAGAAGGGC-3' | 29 |
| 221 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGAGGTCAAAACAGCGTGGATGGCG-3' | 30 |
| 222 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GAGGCTGGATCGGTCCCGGTGTCTT-3' | 31 |
| 223 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AATCCGCGTTCCAATGCACCGTTCC-3' | 32 |
| 224 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TAAAAACTGCGGGCACTGGGGACGG-3' | 33 |
| 225 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT ACCCGAGATTCGCGTGGAGATCCCA-3' | 34 |
| 226 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GAGCAAGGAGCTGCCGAGCGACCAT-3' | 35 |
| 227 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT ACACTGGTGGTGGTGGGCATCGTGC-3' | 36 |
| 228 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TTCCAAATGCGTCAGCGGTGCAAGC-3' | 37 |
| 229 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AGCTGCCTGCATCTTCTTCTGCCGC-3' | 38 |

【００６１】
【表３】

| | | |
|---|---|---|
| 238 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TCTCAGAGGATCGGCCCCCAGAATG-3' | 47 |
| 239 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCTCATCTGACTCCTCGGCGATGGC-3' | 48 |
| 240 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGGGTACAGGGGACTCTGGGGGTGA-3' | 49 |
| 241 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGGTGGGTGCTCTTGCCTCCAGAGG-3' | 50 |
| 100A2 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GACCTCGGGTCGGTAGCACCGCACT-3' | 51 |
| 100C2 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGAAGCCTCTCTTCTCCTCCCCCGG-3' | 52 |
| 100A1 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCACAGACACCGTCCTCACCACCCG-3' | 53 |
| 100B1 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGCTACAGCCACACACGTCTCCTCC-3' | 54 |
| 301 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGAGGCGGGCGGATCACCTGAGGTC-3' | 55 |
| 302 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 56 |
| 320 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TTTTTTTTTTTTTTTTTTTTTTTTTTT-3' | 57 |
| 301A | 5'-CTATTTTTCTATTTTTCTTTT CGAGGCGGGCGGATCACCTGAGGTC-3' | 116 |
| 302C | 5'-CTATTTTTCTATTTTTCTTTT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 117 |
| 302A4 | 5'-CTATTTTATACTTTATATTTCATATTTTATCT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 118 |
| 302A3/2 | 5'-CTATTTTATATTTATATTTCT CGGGAGGCGGAGGTTGCAGTGAGCC ACTATTTTATACTT-3 | 119 |
| 1002A32 | 5'-CTATTTTATACTTTATATTTCT GACCTCGGGTCGGTAGCACCGCAC TACTATTTTATACTT-3' | 120 |
| 301A2/2 | 5'-CTATTTTTCTT CGAGGCGGGCGGATCACCTGAGGTC TTCTTTTTATCTT-3 | 121 |
| 301A3/2 | 5'-CTATTTTATACTTTATATTTCT CGAGGCGGGCGGATCACCTGAGGTC ACTATTTTATACTT-3' | 122 |

【００６２】
【表４】

表2

| プローブ ID | 配列 | 配列 ID |
|---|---|---|
| | 5'-CTATTTTTCTATTTTTCTTTT | 123 |
| | 5'-CTATTTTATACTTTATATTTCATATTTTATCT | 124 |
| 330 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | 58 |
| | 5'-CTATTTTATACTTTATATTTCT.....ACTATTTTATACTT-3 | 125 |
| | 5'-CTATTTTTCTT.....TTCTTTTTATCTT-3 | 126 |

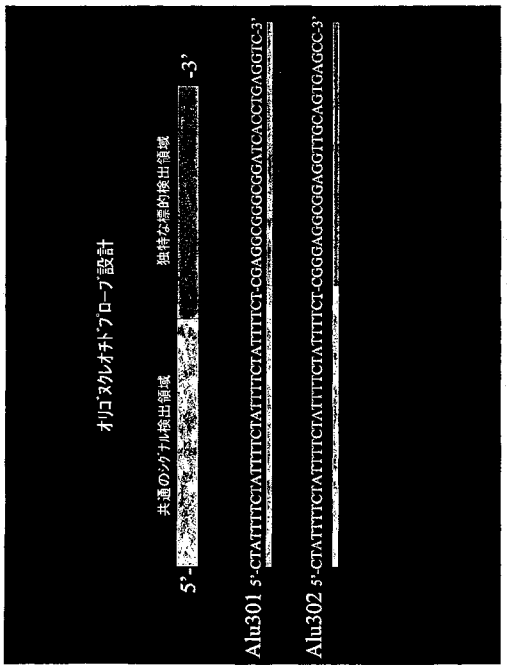
【００６３】
前記の開示は、ある特定の本発明の実施態様を強調しており、そしてあらゆるその改変もしくは代替等価物は、添付される請求項において記述される本発明の精神および範囲内にあることが理解されるべきである。
【図面の簡単な説明】
【図１】
２ドメインプローブ設計の遺伝子プローブ構造を図解する。これは、次の実施例において記述される遺伝子特異的カクテルにおけるプローブのために使用されるオリゴヌクレオチドの設計である。各プローブは、２つのドメイン：５’標識ドメインおよび３’標的遺伝子の標的遺伝子に特異的なドメインからなる。標識ドメインは、この特異配列（ＣＴＡＴＴＴ）$_n$からなり、この場合、シトシンヌクレオチドは、シトシン－ハプテン複合体であり、この実施例ではハプテンはフルオレセインである。この図は、具体的には、３０１（配列番号：５５）および３０２（配列番号：５６）プローブについての核酸配列を示し、これらの各々は、ヒト反復Ａｌｕ配列に対応する標的遺伝子の遺伝子特異的ドメインお

よびフルオレセインハプテンを有する標識ドメインを保持する。
【図２】
標識ドメインを含んでなるプローブ（３３０プローブ；配列番号：５８）を用いるヒト皮膚組織のイン・サイチューハイブリダイゼーション（ＩＳＨ）解析について得られた結果を説明する。検出可能なシグナルの不在は、これらのＩＳＨ実施例において使用されたオリゴヌクレオチドに共通する標識ドメインの配列式（ＣＴＡＴＴＴＴ）$_n$が、それがハイブリダイズしないためにヒト核酸配列とワトソン・クリック塩基対を形成する能力において非特異的で、そして非反応性であることを示している。
【図３】
標識ドメインおよびポリｄ（Ｔ）標的遺伝子特異的ドメインを含んでなるプローブ（３２０プローブ；配列番号：５７）を用いるヒト皮膚組織のＩＳＨ解析について得られた結果を説明する。細胞質に局在される検出可能なシグナルの存在は、このプローブがメッセンジャーＲＮＡのポリアデニル化領域に特異的にハイブリダイズできることを示している。
【図４】
組織サンプルが、イン・サイチューハイブリダイゼーション前にリボヌクレアーゼＡにより処理されなかった（Ａ）か、またはイン・サイチューハイブリダイゼーション前にリボヌクレアーゼＡにより処理された（Ｂ）場合の、３２０プローブを用いるヒト皮膚組織のＩＳＨ解析について得られた結果を説明する。（Ｂ）における検出可能なシグナルにおける減少は、このプローブがメッセンジャーＲＮＡに共通するポリアデニル化領域に特異的にハイブリダイズすることを示している。
【図５】
ハイブリダイゼーションおよびストリンジェンシー洗浄が室温（Ａ）または３７℃（Ｂ）で実施された場合の、３２０プローブを用いるヒト脾臓組織のＩＳＨ解析について得られた結果を説明する。この結果は、より強い発色がより低いストリンジェント条件を示すことによって、発色の強度がハイブリダイゼーション条件のストリンジェンシーに関係することを示している。
【図６】
３２０プローブを用いるヒトＲａｊｉ細胞系のＩＳＨ解析について得られた結果を説明する。これは、このプローブ設計がまた包埋細胞系ならびに包埋組織を用いて機能することを示している。
【図７】
３０１および３０２プローブからなるプローブコレクションを用いるヒトＲａｊｉ細胞系のＩＳＨ解析について得られた結果を説明する。
【図８】
３０１および３０２プローブからなるプローブコレクションを用いるヒトＨＴ細胞系のＩＳＨ解析について得られた結果を説明する。
【図９】
３０１および３０２プローブからなるプローブコレクションを用いるラット細胞系のＩＳＨ解析について得られた結果を説明する。検出可能なシグナルの不在は、このプローブコレクションがヒト核酸配列に特異的であることを示している。
【図１０】
Ｅｐｓｔｅｉｎ－Ｂａｒｒウイルス（ＥＢＶ）　ＥＢＥＲ核ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブ［配列番号：５１～配列番号：５４］を用いるＥＢＶ陰性ヒトＨＴ細胞系のＩＳＨ解析について得られた結果を説明する。
【図１１】
ＥＢＶ　ＥＢＥＲ１および２核ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：５１～配列番号：５４］を用いるヒト脾臓組織のＩＳＨ解析について得られた結果を説明する。
【図１２】
ＥＢＶ　ＥＢＥＲ１および２核ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプ

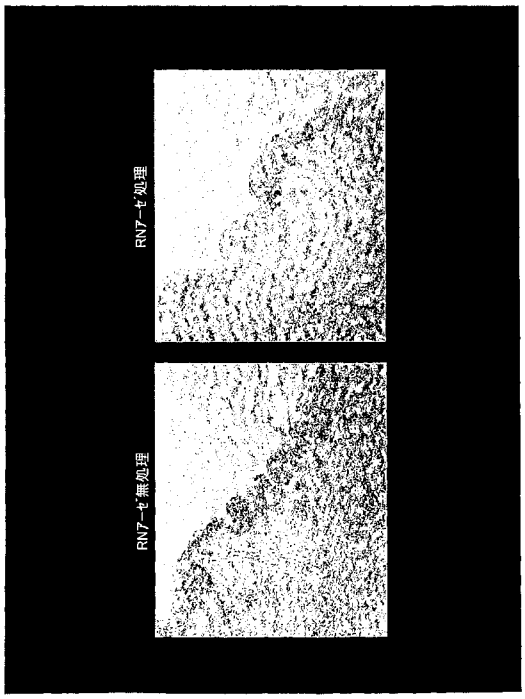
ローブからなるプローブコレクション［配列番号：５１～配列番号：５４］を用いるヒト扁桃組織のＩＳＨ解析について得られた結果を説明する。
【図１３】
ＥＢＶ　ＥＢＥＲ１および２核ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：５１～配列番号：５４］を用いるヒト脾臓組織のＩＳＨ解析について得られた結果を説明するが、この場合、組織サンプルは、イン・サイチューハイブリダイゼーション前にリボヌクレアーゼＡにより処理されなかった（Ａ）か、またはイン・サイチューハイブリダイゼーション前にリボヌクレアーゼＡにより処理された（Ｂ）。（Ｂ）における検出可能なシグナルにおける減少は、このプローブがヒトＥＢＥＲ１および２核ＲＮＡに特異的にハイブリダイズすることを示している。
【図１４】
ヒト免疫グロブリンλ軽鎖ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブ［配列番号：１５］を用いるκ軽鎖ポジティブヒト扁桃組織のＩＳＨ解析について得られた結果を説明する。
【図１５】
ヒト免疫グロブリンλ軽鎖ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：２－４、配列番号：７－１２、配列番号：１４，１５］を用いるリンパ腫組織のＩＳＨ解析について得られた結果を説明する。（Ａ）のリンパ腫組織はκ軽鎖を過発現し、そして（Ｂ）の組織はλ軽鎖を過発現する。（Ｂ）における検出可能なシグナルの不在は、κ軽鎖プローブコレクションがκ軽鎖ｍＲＮＡに特異的であることを示している。
【図１６】
ヒト免疫グロブリンλ軽鎖可変部ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブ［配列番号：１９－２９］を用いるλ軽鎖ポジティブヒト扁桃組織のＩＳＨ解析について得られた結果を説明する。
【図１７】
ヒト免疫グロブリンλ軽鎖ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：１９－２９］を用いるλ軽鎖ポジティブヒトＲＰＭＩ　８２２６細胞系のＩＳＨ解析について得られた結果を説明する。
【図１８】
ヒト免疫グロブリンλ軽鎖ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：１９－２９］を用いるヒト脾臓組織のＩＳＨ解析について得られた結果を説明する。（Ａ）の組織はλ軽鎖を過発現し、そして（Ｂ）の組織はκ軽鎖を過発現する。（Ｂ）における検出可能なシグナルの不在は、λ軽鎖プローブコレクションがヒトλ軽鎖ｍＲＮＡに特異的であることを示している。
【図１９】
ＣＭＶ即時型ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：３０－３２，配列番号：３４－３５，配列番号：３８，配列番号：５０］を用いるサイトメガロウイルス（ＣＭＶ）ポジティブヒト肺組織のＩＳＨ解析について得られた結果を説明する。矢印はＣＭＶ感染細胞を示す。
【図２０】
ＣＭＶ即時型ｍＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：３０－３２，配列番号：３４－３５，配列番号：３８，配列番号：５０］を用いる、ＣＭＶ即時型ＲＮＡの発現がシクロヘキサミドによって誘発されなかったラット９Ｇ細胞系のＩＳＨ解析について得られた結果を説明する。
【図２１】
ＣＭＶ即時型ＲＮＡに対応する標的遺伝子特異的ドメインを保持するプローブからなるプローブコレクション［配列番号：３０－３２，配列番号：３４－３５，配列番号：３８，配列番号：５０］を用いる、ＣＭＶ即時型ＲＮＡの発現がシクロヘキサミドによって誘発されたラット９Ｇ細胞系のＩＳＨ解析について得られた結果を説明する。（Ａ）の組織は

倍率４０Ｘにおいて示され、そして（Ｂ）の組織は倍率２０Ｘにおいて示される。

【図１】




【図７】



【図１２】

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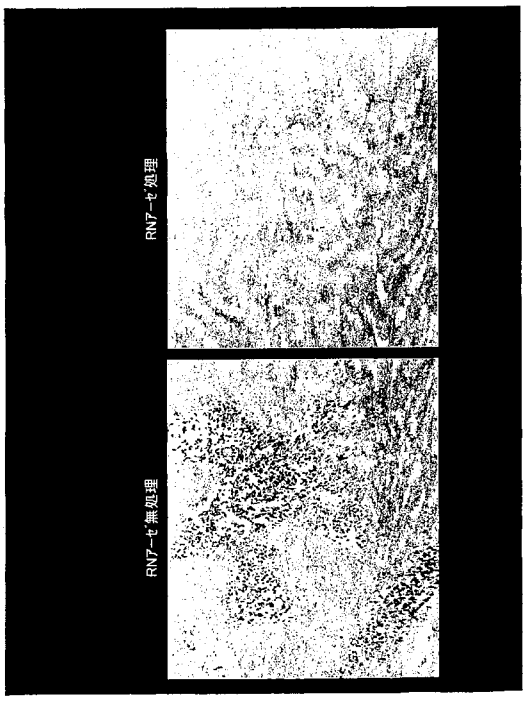


【図１５】

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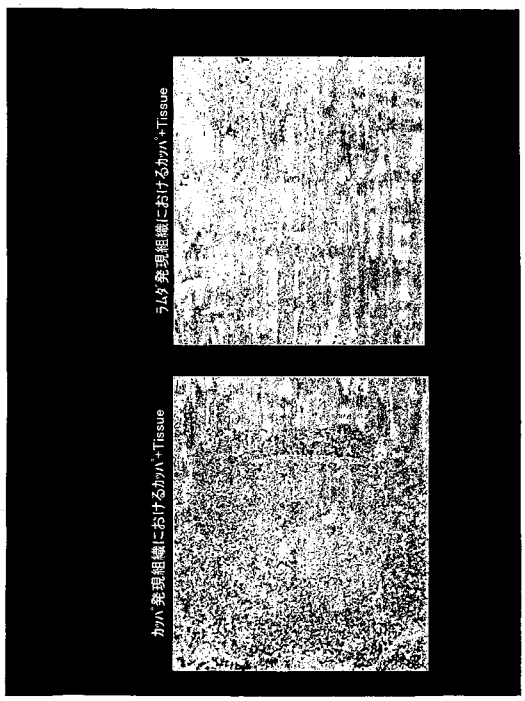


【図１８】

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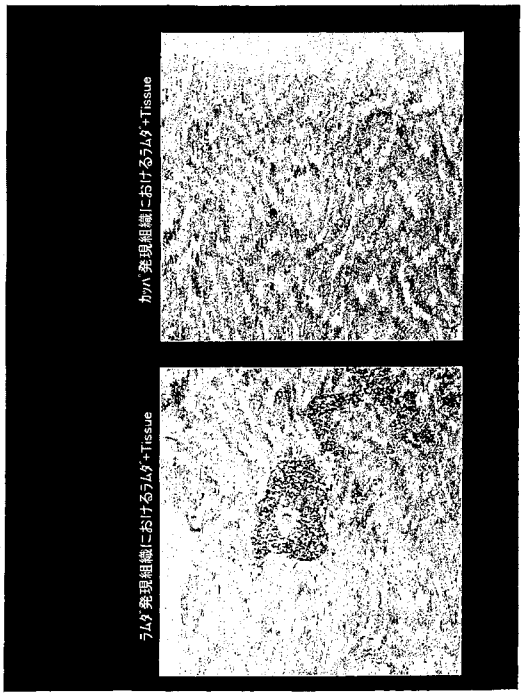

【国際公開パンフレット】

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 March 2002 (21.03.2002)

PCT

(10) International Publication Number
WO 02/22874 A2

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number: PCT/US01/28014

(22) International Filing Date:
6 September 2001 (06.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/233,177 15 September 2000 (15.09.2000) US

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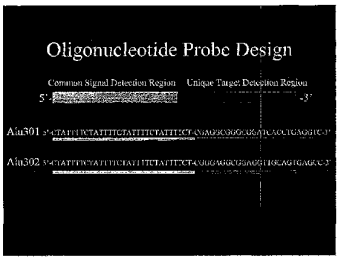
(81) Designated States *(national)*: AU, CA, JP, MX, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *without international search report and to be republished upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: OLIGONUCLEOTIDE SEQUENCE FORMULA FOR LABELING OLIGONUCLEOTIDE PROBE AND PROTEINS FOR IN-SITU ANALYSIS




(57) Abstract: The present invention provides oligonucleotide probes and oligonucleotide probe collections and protein labeling for detecting or localizing a plurality nucleic acid target genes or antigens within a cell or tissue sample. Specifically, the provides collections of oligonucleotide probe for use in *in situ* hybridization analyses in which each probe has a label-domain with the sequence formulas of $(CTATTTT)_n$, $(AAAATAG)_n$ or $(TTTTATC)_n$ or $(GATAAAA)_n$ in which all cases "n" would equal 1 or greater. The present invention provides collections or "cocktails" of oligonucleotide probes for detecting or localizing specific nucleic acid target genes within a cell or tissue sample. The cocktails are useful for detecting the following: the Kappa gene (SEQ ID NOS: 1-16 inclusive); the Lamba gene (SEQ ID NOS: 501-509, 511-513, and 515); the CMV (cytomegalovirus) gene (SEQ ID NOS: 221-241 inclusive); EBER (Epstein-Barr RNA) gene (SEQ IN NOS: 51-54 inclusive): Alu (SEQ IN NOS: 55-56); PolyA (SEQ ID NO: 57); and the detection tail (SEQ ID NO: 330).

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## BACKGROUND OF THE INVENTION

### 1. Field of the Invention

This invention relates to oligonucleotide probes and collections of oligonucleotide probes for detecting or localizing nucleic acid genes targets witin a cell or tissue sample. In particular, the invention relates to collections of oligoprobes.

### 2. Background of the Invention

*In situ* analysis includes in situ hybridization and immunohistochemistry. *In situ* hybridization (ISH) employs labeled DNA or RNA probe molecules that are anti-sense to a target gene sequence or transcript to detect or localize targeted nucleic acid target genes within a cell or tissue sample. ISH has proven to be a useful tool in a number of biomedical fields, including developmental biology, cell biology, and molecular biology. ISH has been used, for example, to diagnose genetic disorders, map genes, study gene expression, and localize sites of target gene expression.

Typically, ISH is performed by exposing a cell or tissue sample immobilized on a glass slide to a labeled nucleic acid probe which is capable of specifically hybridizing to a given target gene in the cell or tissue sample (*In Situ Hybridization: Medical Applications* (G. R. Coulton and J. de Belleroche, eds., Kluwer Academic Publishers, 1992); *In Situ Hybridization: In Neurobiology; Advances in Methodology* (J. H. Eberwine, K. L. Valentino, and J. D. Barchas, eds., Oxford University Press, 1994); *In Situ Hybridization: A Practical Approach* (D. G. Wilkinson, ed., Oxford University Press, 1992)). The hybridization of labeled probe molecules to nucleic acids in the cell or tissue sample can then be detected using, for example, radioactive-based direct detection methods, fluorescence-based direct detection methods, or indirect detection methods based on the binding of a fluorescence-labeled protein binding to a hapten such as BrdU, digoxigenin-labeled or biotin-labeled nucleotides incoporated into probes. Hapten-based methods have been further extended to include those molecules to be bonded by binding protein-enzyme conjugates such as antibody-enzyme-conjugates and colorimetric based detection chemistry. In addition, several target genes can be simulanteously analyzed by exposing a cell or tissue sample to a plurality of nucleic acid probes that have been labeled with a plurality of different nucleic

acid tags. For example, a plurality of nucleic acid probes can be labeled with a plurality of fluorescent compounds having different emission wavelengths, thereby permitting simultaneous multicolored analysis to be performed in a single step on a single target cell or tissue sample.

A significant problem associated with incorporation of labeled nucleotides into oligonucleotide probes is that the conjugation moieties that are attached to the nucleotide usually interfere with the formation of Watson-Crick base pairing, thus negatively affecting the hybridization of the probe to its target. The has been seen with use of label attached via N4-substitued cytosine nucleotides, because of steric hinderance and the expected shift to the less reactive state of a secondary amine (as seen with N4 labled cytosine), as compared to the natural G-C bond formed with an unsubstituted cytosine (a primary amine). Any small change or interference with G-C bonding in a small oligonucleotide (25 to 50 bases) can reduce the ability of these oligos to hybridize with the intended targeted sequence.

There remains a need in the art to develop suitable probes designs for incorporating labeled nucleotides in oligonucleotide probes. We demonstrate that a few artificial seqeunces are viable alternatives for probe labeling and also work both singly and in complex oligonucleotide probe mixtures for detecting or localizing nucleic acid target genes within a cell or tissue sample. The development of such generic seqeunces and labeling strategy for probe collections has wide application in the medical, genetic, and molecular biological arts.

This interference due to labeling chemistry and hybridization stringency and kinetics is solved herein by designing the oligo to have at least two distinct functional domains, one domain or sequence to be gene specific and involved in the base pair formation, and the second domain to be an artificial, non-specific sequence (in reference to the sample's genome) comprised of spacing nucleotides and the labeled nucleotide. These elements are positioned so that these label-nucleotides are more accessible as haptens for binding proteins (immunoglobulin or avidin(s)) and thus do not interfere with Watson-Crick base pairing in the gene-specific domain.

## SUMMARY OF THE INVENTION

The present invention provides a novel strategy to incorporate label into oligonucleotide probes and labeled oligonucleotide probe collections for detecting or

localizing nucleic acid target genes within a cell or tissue sample. In particular, the invention relates to non-gene-specific sequences using sequence formulas for making repetitive polymers of such sequences which can be incorporated into collections of oligonucleotide probes for use in *in situ* hybridization analyses. In addition, using labeled synthetic oligonucleotide polymers, based on sequence formulas, when conjugated to binding proteins, i.e. immunglobulins, is a very effective and controlled process for labeling such proteins used in immunohistochemical analysis. The present invention provides collections or "cocktails" of oligonucleotide probes for detecting or localizing specific nucleic acid target genes within a cell or tissue sample. The cocktails are useful for detecting the following: the Kappa gene (SEQ ID NOS: 1-16 inclusive); the Lamba gene (SEQ ID NOS: 501-509, 511-513, and 515); the CMV (cytomegalovirus) gene (SEQ ID NOS: 221-241 inclusive); EBER (Epstein-Barr early RNA) gene (SEQ ID NOS: 51-54 inclusive); Alu (SEQ ID NOS: 55-56); PolyA (SEQ ID NO: 57); and the detection tail (SEQ ID NO:330).

The invention is directed to an oligonucleotide label-domain comprising the sequence $(CTATTTT)_n$ and its complement $(AAAATAG)_n$ wherein "n" is at least 1.

The invention is also directed to an oligonucleotide probe having at least two distinct functional domains, a first domain comprising the label-domain of claim 2, and a second domain comprising a gene-specific target sequence.

The invention is also directed to a probeset for detecting Kappa immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 401 through 416, inclusive.

The invention is also directed to a probeset for detecting Lambda immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 501 through 509, 511-513, and 515.

The invention is also directed to A probeset for detecting cytomegalovirus (CMV) immediate early RNA and /or corresponding mRNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 221 through 241

The invention is also directed to a probeset for detecting Epstein Barr virus (EBV) early RNA, RNA 1 and RNA 2, (EBER) wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 51 through 54.

The invention is also directed to a probeset for detecting Human Alu repetitive sattelite genomic DNA sequences wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 301 and 302.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a generic probe structure of of the two-domain probe design. This is the oligonucleotide design used for the probes in the gene specific cocktails described in the following examples. Each probe is composed of two domains: a 5' labeling domain and a 3' target gene target gene-specific domain. The labeling domain consists of this specific sequence (CTATTTT)n, wherein each cytosine may be labeled with a fluorophore or a cytosine-hapten conjugate, the hapten being fluorescein in this embodiment. This illustration specifically shows nucleic acid sequences for the 301 (SEQ ID NO: 55) and 302 (SEQ ID NO: 56) probes, each of which possesses target gene gene-specific domains corresponding to human repetitive Alu sequences and labeling domains having a fluorescein hapten.

Figure 2 illustrates the results obtained for *in situ* hybridization (ISH) analysis of human skin tissue using a probe comprising the labeling domain (330 probe; SEQ ID NO: 58). The absence of a detectable signal indicates that the sequence formula, (CTATTTT)n, of the labeling domain common to the oligonucleotides used in these ISH examples is non-specific, and non-reactive in its ability to form Watson-Crick base pairing with human nucleic acid sequences because it does not hybridize.

Figure 3 illustrates the results obtained for ISH analysis of human skin tissue using a probe comprising the labeling domain and a poly d(T) target gene-specific domain (320 probe; SEQ ID NO: 57). The presence of a detectable signal localized to the cytoplasm

indicates that this probe is capable of specifically hybridizing to polyadenylated region of messenger RNA.

Figures 4A-4B illustrate the results obtained for ISH analysis of human skin tissue using the 320 probe, wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The decrease in detectable signal in (B) indicates that this probe specifically hybridizes to polyadenylated region common to messenger RNA. .

Figures 5A-5B illustrate the results obtained for ISH analysis of human spleen tissue using the 320 probe, wherein the hybridization and stringency wash were performed at room temperature (A), or at 37°C (B). This result illustrates that the intenstity of color is related to the stringency of hybridization conditions, with the more intense color indicating less stringent conditons.

Figure 6 illustrates the results obtained for ISH analysis of the human Raji cell line using the 320 probe. This shows that this probe design also is functional with embedded cell lines as well as embedded tissue.

Figure 7 illustrates the results obtained for ISH analysis of the human Raji cell line using a probe collection consisting of the 301 and 302 probes.

Figure 8 illustrates the results obtained for ISH analysis of the human HT cell line using a probe collection consisting of the 301 and 302 probes.

Figure 9 illustrates the results obtained for ISH analysis of a rat cell line using a probe collection consisting of the 301 and 302 probes. The absence of a detectable signal indicates that this probe collection is specific for human nucleic acid sequences.

Figure 10 illustrates the results obtained for ISH analysis of an Epstein-Barr virus (EBV)-negative human HT cell line using a probe possessing a target gene-specific domain corresponding to EBV EBER nuclear RNA [SEQ ID NO: 51 through SEQ ID NO: 54?].

Figure 11 illustrates the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figure 12 illustrates the results obtained for ISH analysis of human tonsil tissue using a probe collection consisting of probes possessing target gene-specific domains

corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figures 13A-13B illustrate the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54, wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The decrease in detectable signal in (B) indicates that this probe specifically hybridizes to human EBER 1 and EBER 2 nuclear RNA.

Figure 14 illustrates the results obtained for ISH analysis of kappa light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NO:15].

Figure 15 illustrates the results obtained for ISH analysis of lymphoma tissues using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin kappa light chain mRNA [SEQ ID NOS: 2-4, SEQ ID NOS:7-12, SEQ ID NOS: 14, 15]. The lymphoma tissue in (A) over expresses the kappa light chain and the tissue in (B) over expresses the lambda light chain. The absence of a detectable signal in (B) indicates that the kappa light chain probe collection is specific to kappa light chain mRNA.

Figure 16 illustrates the results obtained for ISH analysis of lambda light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain variable region mRNA [SEQ ID NOS:19 through 29].

Figure 17 illustrates the results obtained for ISH analysis of a lambda light chain-positive human RPMI 8226 cell line using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NOS:19 through 29].

Figures 18A-18B illustrate the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NOS:19 through 29]. The tissue in (A) over expresses the lambda light chain and the tissue in (B)

over expresses the kappa light chain. The absence of a detectable signal in (B) indicates that the lambda light chain probe collection is specific to human lambda light chain mRNA.

Figure 19 illustrates the results obtained for ISH analysis of cytomeglovirus (CMV)-positive human lung tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [ SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50]. [ CMV infected cell ]

Figure 20 illustrates the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has not been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early mRNA [ SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50].

Figures 21A-21B illustrate the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50. The tissue in (A) is shown at a magnification of 40X and the tissue in (B) is shown at a magnification of 20X.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

The present invention provides oligonucleotide probes and oligonucleotide probe collections for detecting or localizing nucleic acid target genes within a cell or tissue sample. In particular, the invention relates to collections of oligonucleotide probes for use in *in situ* hybridization analyses.

More specifically, this invention relates to the use of specific sequence formulas for nucleotide polymers or label-domains to attach a detectable moity ( a label) to oligonucleotide probes or proteins. The specific utility of these seqeuces or derivatives thereof, is the inert or non-reactive characteristic that does not hybridize to human DNA or RNA at a detectable level under standard stringency of hybridization conditions. These label-domains or polymers were demonstrated to be useful generic sequences for incorporation into oligonucleotide probes for detecting gene-specific sequences within cells or tissue samples in in situ hybridizaiton analyses. Additionally, this inert set of sequences

are useful for attaching a label to immunoglobulins or other proteins for detecting haptens and antigens in immunohistochemical analyses.

As used herein, the terms "probe" or "oligonucleotide probe" refers to a nucleic acid molecule used to detect a complementary nucleic acid target gene.

As used herein, the term "hybridization" refers to the process whereby complementary nucleic acid sequences join to form a double-stranded nucleic acid molecule. By labeling the target nucleic acid molecule with, for example, a radioactive or fluorescent tag, interactions between probe and target genes can be detected.

The oligonucleotide probes and oligonucleotide probes of the collections of the present invention are synthesized using conventional methods. *See e.g., Methods in Molecular Biology, Vol 20: Protocols for Oligonucleotides and Analogs* 165-89 (S. Agrawal, ed., 1993); *Oligonucleotides and Analogues: A Practical Approach* 87-108 (F. Eckstein, ed., 1991).

In a preferred embodiment of the present invention, oligonucleotide probes possess two distinct domains: a 5' (or labeling) domain and a 3' (or gene-specific target) domain (*See* Figure 1A). In more preferred embodiments of the present invention, the oligonucleotide probe possesses a labeling domain which consists of the sequence (CTATTTT)n. Other embodiments are also demonstrated herein, including a triple-domain embodiment having two terminal labeling domains, and a central gene-specific target domain. Specifically, SEQ ID NOS: 125-126 depict this labeling scheme. Yet a further preferred embodiment of a labeling domain is TC(TTTTATC)$_n$ or its complement. This sequence is predicted to be as unique as the (CTATTTT)n label-domain. The oligonucleotide probes of the present invention are labeled so that hybridization between said probes and target nucleic acids in a particular cell or tissue can be detected. Labels that are acceptable for use in *in situ* hybridization (ISH) analysis are known to those with skill in the art. Such labels permit interactions between probe and target genes to be detected using, for example, radioactive-based direct detection methods, fluorescence-based direct detection methods, digoxigenin-labeled or biotin-labeled probes coupled with fluorescence-based detection methods, or digoxigenin-labeled or biotin-labeled probes coupled with antibody-enzyme-based detection methods. In perferred embodiments of the present invention, oligonucleotide probes are labeled with fluorescein. In more preferred embodiments of the

present invention, the oligonucleotide probe possesses a labeling domain which consists of the sequence (CTATTTT)n, wherein the cytosine nucleotides may be labeled with a flurophore for direct detection, or a hapten for indirect detection. In either, the fluorescein-cytosine nucleotide conjugate and the fluorescein molecule is linked at the N4 position of cytosine through an OBEA linkage (*See* Mishra *et al.*, U.S. Patent No. 5,684,142, which is incorporated herein by reference). In a preferred embodiment, the density of fluorphore attached to the label-domain is at least 7 mole percent, preferably at least 10 mole percent, and most preferably at least 16 mole percent, when measured aginst the label-domain solely. For example, if probe 401 is considered (a 2-domain probe) it comprises a label-domain of 30 bases including a 3' terminal CT wherein the C is also labeled, the mole percent is 5/30 = 16.7 mole percent label. In the overall probe, the mole percent is 8.3.

In some embodiments of the present invention, several target genes are simulanteously analyzed by exposing a cell or tissue sample to a plurality of nucleic acid probes that have been labeled with a plurality of different nucleic acid tags. For example, a plurality of nucleic acid probes can be labeled with a plurality of fluorescent compounds having different emission wavelengths, thereby permitting simultaneous multicolored analysis to be performed in a single step on a single target cell or tissue sample.

The oligonucleotide probes and oligonucleotide probe collections of the present invention may be used in ISH analysis to detect or localize nucleic acid target genes within a cell or tissue sample. ISH may be performed as described, for example, in *In Situ Hybridization: Medical Applications* (G. R. Coulton and J. de Belleroche, eds., Kluwer Academic Publishers, 1992); *In Situ Hybridization: In Neurobiology; Advances in Methodology* (J. H. Eberwine, K. L. Valentino, and J. D. Barchas, eds., Oxford University Press, 1994); or *In Situ Hybridization: A Practical Approach* (D. G. Wilkinson, ed., Oxford University Press, 1992)).

The preferred embodiment of the probes and probe collections of the present invention are best understood by referring to Figures 1-21 and Examples 1-2. The Examples, which follow, are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

**EXAMPLE 1**

**Probe Collection Preparation**

Probe collections consisting of a plurality of oligonucleotide probes of 55 to 60 bases in length were designed as follows. In this Example, each oligonucleotide probe possessed two distinct domains: a 5' (or labeling) domain and a 3' (or target gene-specific) domain (*See* Figure 1).

In this embodiment, the labeling domain consists of the sequence (CTATTTT)n, wherein the cytosine nucleotide represents a fluorescein-cytosine nucleotide conjugate and the fluorescein molecule is linked at the N4 position of cytosine through an OBEA linkage.

The target gene-specific domain consists of a 25-30 base sequence that is complementary to a specific nucleic acid target gene. Oligonucleotide probes were designed to possess target gene-specific domains corresponding to the human immunoglobulin kappa light chain variable region (*See* Table 1; oligonucleotide probes 401-416), the human immunoglobulin lambda light chain variable region (oligonucleotide probes 501-515), human cytomegalovirus (CMV) sequences (oligonucleotide probes 221-241), human Esptein-Barr virus (EBV) EBER (Epstein-Barr early RNA) sequences (oligonucleotide probes 100A2, 100C2, 100A1, and 100B1), human repetitive Alu sequences (oligonucleotide probes 301 and 302), and poly d(T) (oligonucleotide probe 320).

**EXAMPLE 2**

**Label-domain design: Alu repetitive sequence probe**

Four probes all against the Alu human repetative sequence were used to evaluate label-domain design. The probes numbered 301 (SEQ ID NO: 55), 301A (SEQ ID NO:116), 301A2/2 (SEQ ID NO: 121), and 301A3/2 (SEQ ID NO: 122) are shown in Table 1.

The four probes were evaluated at the concentrations of 100, 75, 50, and 25 ng/ml per mL of probe in the reaction, respectively. This hybridization analysis was done manually, using standard protocols. The target, paraffin-embedded cell line MBA MD 468 (Oncor INFORM™ Her-2/neu Control Slides, Cat. No. S8100, Level 1, available from Ventana Medical Systems, Inc., Tucson, AZ) was the target sample and was processed by removing paraffin by standard xylene methods. The tissue was subjected to Ventana's. Protease 1 for 12 minutes at 50 degrees C as a 1:2 dilution with Ventana's APK buffer. The hybridization reaction was accomplished with the addition of probe diluent as 100 ul probe (25%

formamide, 5% dextran sulfate, 2X SSC, 1% Triton) to a residual 100 ul volume of 2XSSC/Triton X-100. The slide was heated to 85 degrees C for 5 minutes and then incubated for 1 hr at 37 degrees C. Standard SSC washes followed for removing excess probe. The hybrids were detected with an antibody against FITC. The mouse antibody was detected colormetrically using Ventana Enhanced Alkaline Phosphatase Blue Detection (cat# 760-061). Unless otherwise indicated, all reagents were obtained from Ventana Medical Systems, Inc., Tucson, AZ. The results were observed by colormetric detection using brightfield microscopy.

The results of these experiments were that signal intensity was a function of the total number of fluorescein hapten conjugated to the probe and signal was of the specific label-domain design. The greater the number of fluoresceins per probe molecule, the greater the signal observed. Comparison of design and placement of haptens on the probe showed that this was not a factor in signal intensity. The two probes that contained five fluoresceins, (301A3/2 (SEQ ID NO: 122) and 301 (SEQ ID NO:55) both yielded equivalent signal. These two probes yielded greater signal that seen for 301A2/2, a probe with a split label-domain design with four fluoresceins. The probe 301A2/2 yielded a signal greater than probe 301A a probe with a single label-domain design at the 5' end and with three fluoresceins.

**EXAMPLE 3**

**Label Domain Design: EBER probes**

This experiment compared two label-domain designs and sequences to determine whether greater spacing between the fluorescein haptens improves the production of signal during probe detection steps during *in situ* hybridization analysis.

The tissue used was an EBV-infected human spleen tissue fixed in neutral buffered formalin paraffin embedded section of 4-micron thickness placed on silane plus glass microscope slides. The tissue sections were deparaffinized on a Ventana DISCOVERY™ machine, followed by a 6-min digestion with Ventana's Protease 1, at a temperature of 37 C. The probe was dissolved in hybridization buffer diluent at a concentration of 50 ng/mL as a 100 ul applied to an equal volume of 2 X SSC/ Triton X-100 residual volume left on the slide after prepared by the Ventana Medical Systems, Inc. automated ISH staining system, Discovery. The probe diluent-mixed with the residual volume on slide for 6 min at 37 C,

then the solution was heated to 85C and held there for a total of 10 min. The slide was then taken to a 37C temperature and held at that temperature for 1 hour. All of these aqueous reactions on the slide were all done under a film of LIQUID COVERSLIP™, to prevent evaporative loss of water during processing. Each slide after hybridization was washed 3 times with 2X SSC/Triton solution, with a 6 min incubation between each wash, the slide volume being approximately 300 ul (+/- 10% vol). The hybrids were detected with an antibody against FITC. The mouse antibody was detected colormetrically using Ventana Enhanced Alkaline Phosphatase Blue Detection (cat# 760-061).

The two oligonucleotide probes used for this study probe 100A1 (SEQ ID NO: 53) and 1002A32 (SEQ ID NO: 120). The two differences between these probes were the label-domain seqence and structure. The probe 100A1 label domain was 5' to gene target domain, contained 5 fluoresceins attached to cytosine residues via the OBEA linker, with the sequence formula of $(CTATTTT)_4CT$ (SEQ ID NO: 58). The label domain of the oligo probe 1002A32, was similiar, (SEQ ID NO:125). Besides the different sequence the primary difference was that the fluoresceine labeled cytosines were spaced 10 bases apart compared to the oligo 100A1 the cytosine spacing was closer at 7 bases apart. The result of this comparison as deduced by H score analysis were that these oligonucleotide were equivalent as to the amount of signal generated on the slide. The data was that for 100A2, for the 368 cells analysed in a total of 3 fields the H score was 106, and for probe 1002A32 for the 345 cell analysed in three field the H score was 109. The H score is a spectrographic analysis done with micrscope that factors into the score background to signal ratio on the tissue section to yield a relative comparison of total target specific signal on the slide. (See reference Giroud, F. Perrin C, and Simony Lafontaine, J.; Quantitative Immunocytochemistry and Immunohistochemistry. Third Conference of the European Society for Analytical Cellular Pathology, 1994; and AutoCyte Quic Immuno User's Manual, 1998, document number PA-029, Co AutoCyte Inc. Burlington NC 2721). The histograms and the score sheet indicated that each oligo were equally efficient in yielding a colormetric signal. This indicates that the position of the label domain can be either 3 prime or 5 prime to the gene target sequence or the gene target sequence can be positioned between two label domains.

**EXAMPLE 4**

***In Situ* Hybridization**

The probe collections prepared in Example 1 were first diluted in a solution consisting of 20% dextran sulfate (wt/vol), 50% formamide (vol/vol), 2X SSC, 10 mM Tris-HCl, 5 mM EDTA, and 0.05% Brij-35, at a final pH of 7.3. Probe collections were then mixed with an equal volume of a solution consisting of 2X SSC and 0.05% Triton X-100.

Samples for ISH analysis were prepared by cutting formalin-fixed and paraffin-embedded cells or tissue samples into 4 μm sections and placing the sections onto a glass slide. Subsequent processing and ISH of samples was carried out in an automated device, such as the DISCOVERY™ Automated ISH/IHC Stainer (Ventana Medical Systems, Inc., Tucson, AZ) described in co-owned and co-pending U.S. Patent App. Serial Nos. 60/076,198 and 09/259,240, both incorporated herein by reference. . To remove paraffin from the samples, the slides were immersed in an aqueous solution, heated for approximately 20 minutes, and then rinsed. The automated deparaffinization procedure is more fully described in U.S. Serial No. 60/099,018, 09/259,240 both incorporated herein by reference. The samples were then treated with protease and the slides were heated to 85°C (for hybridization to RNA target genes) or 90-95°C (for hybridization to DNA target genes) for 4 to 10 minutes.

Hybridization reactions were typically performed in a hybridization buffer consisting of 10% dextran sulfate (wt/vol), 25% formamide (vol/vol), 2X SSC, 5 mM Tris, 2.5 mM EDTA, 0.025% Brij-35, 0.25% Triton X-100, and between 25 to 125 ng/mL of each individual probe molecule. ISH reactions were performed at between 37°C to 54°C. For ISH using the probe collections described in Example 1, hybridization reactions were optimally carried out for 1 hr at 47°C (except for the poly d(T) probe, wherein the hybridization reaction was optimally carried out at 37°C for 1 hr).

The hybridization of fluorescein-labeled probe molecules to a particular target gene in the sample was detected by using a sequential series of binding proteins, i.e., secondary antibody detection. However, it is equally possible to use detect detection when visualizing the bound probes. In secondary detection, first, an anti-fluorescein mouse monoclonal antibody directed against the fluorescein-labeled probe molecule was added to the sample. Next, a biotin-labeled polyclonal goat antibody directed against the mouse antibody was added to the sample. Finally, hybridization reactions were colormetrically detected using a 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) substrate This technique, termed "secondary antibody detection," is routine for one of skill in the art.

Primary and secondary antibodies are available from numerous suppliers, including Ventana Medical Systems, Tucson, AZ, which are optimized for use on the Ventana autostaining systems (ES®, NexES®, DISCOVERY™, and BENCHMARK™).

Figures 2-21 illustrate the results obtained for *in situ* hybridization analysis of various cell lines or tissue samples using the probes disclosed and claimed herein having the structural motif illustrated in Figure 1 or probe collections consisting of such probes.

Figure 1 illustrates a generic probe structure of of the two-domain probe design. This is the oligonucleotide design used for the probes in the gene specific cocktails described in the following examples. Each probe is composed of two domains: a 5' labeling domain and a 3' target gene target gene-specific domain. The labeling domain consists of this specific sequence (CTATTTT)n, wherein the cytosine nucleotide is a cytosine-hapten conjugate, the hapten being fluorescein in this embodiment. This illustration specifically shows nucleic acid sequences for the 301 (SEQ ID NO: 55) and 302 (SEQ ID NO: 56) probes, each of which possesses target gene gene-specific domains corresponding to human repetitive Alu sequences and labeling domains having a fluorescein hapten.

Figure 2 illustrates the results obtained for *in situ* hybridization (ISH) analysis of human skin tissue using a probe comprising the labeling domain (330 probe; SEQ ID NO: 58). The absence of a detectable signal indicates that the sequence formula, (CTATTTT)n, of the labeling domain common to the oligonucleotides used in these ISH examples is non-specific, and non-reactive in its ability to form Watson-Crick base pairing with human nucleic acid sequences because it does not hybridize.

Figure 3 illustrates the results obtained for ISH analysis of human skin tissue using a probe comprising the labeling domain and a poly d(T) target gene-specific domain (320 probe; SEQ ID NO: 57). The presence of a detectable signal localized to the cytoplasm indicates that this probe is capable of specifically hybridizing to polyadenylated region of messenger RNA.

Figures 4A-4B illustrate the results obtained for ISH analysis of human skin tissue using the 320 probe, wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The decrease in detectable signal in (B) indicates that this probe specifically hybridizes to polyadenylated region common to messenger RNA. .

Figures 5A-5B illustrate the results obtained for ISH analysis of human spleen tissue using the 320 probe, wherein the hybridization and stringency wash were performed at room temperature (A), or at 37°C (B). This result illustrates that the intenstity of color is related to the stringency of hybridization conditions, with the more intense color indicating less stringent conditons.

Figure 6 illustrates the results obtained for ISH analysis of the human Raji cell line using the 320 probe. This shows that this probe design also is functional with embedded cell lines as well as embedded tissue.

Figure 7 illustrates the results obtained for ISH analysis of the human Raji cell line using a probe collection consisting of the 301 and 302 probes.

Figure 8 illustrates the results obtained for ISH analysis of the human HT cell line using a probe collection consisting of the 301 and 302 probes.

Figure 9 illustrates the results obtained for ISH analysis of a rat cell line using a probe collection consisting of the 301 and 302 probes. The absence of a detectable signal indicates that this probe collection is specific for human nucleic acid sequences.

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Figure 12 illustrates the results obtained for ISH analysis of human tonsil tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figures 13A-13B illustrate the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54], wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The

decrease in detectable signal in (B) indicates that this probe specifically hybridizes to human EBER 1 and EBER 2 nuclear RNA .

Figure 14 illustrates the results obtained for ISH analysis of kappa light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NO:15].

Figure 15 illustrates the results obtained for ISH analysis of lymphoma tissues using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin kappa light chain mRNA [SEQ ID NOS: 2 - 4, SEQ ID NOS:7-12, SEQ ID NOS: 14, 15]. The lymphoma tissue in (A) over expresses the kappa light chain and the tissue in (B) over expresses the lambda light chain. The absence of a detectable signal in (B) indicates that the kappa light chain probe collection is specific to kappa light chain mRNA.

Figure 16 illustrates the results obtained for ISH analysis of lambda light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain variable region mRNA [SEQ ID NOS:19 through 29].

Figure 17 illustrates the results obtained for ISH analysis of a lambda light chain-positive human RPMI 8226 cell line using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NOS:19 through 29].

Figures 18A-18B illustrate the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NOS:19 through 29]. The tissue in (A) over expresses the lambda light chain and the tissue in (B) over expresses the kappa light chain. The absence of a detectable signal in (B) indicates that the lambda light chain probe collection is specific to human lambda light chain mRNA.

Figure 19 illustrates the results obtained for ISH analysis of cytomeglovirus (CMV)-positive human lung tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50]. Arrow indicates CMV infected cell.

Figure 20 illustrates the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has not been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50].

Figures 21A-21B illustrate the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50] expression of the CMV immediate early RNA with cyclohexamide. The tissue in (A) is shown at a magnification of 40X and the tissue in (B) is shown at a magnification of 20X.

**Table 1**

| Probe ID | Sequence | SEQ ID |
|---|---|---|
| 401 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCAGAGTAGCAGGAGCCCCAGGAGCTGAGC-3' | 1 |
| 402 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGATGGAGACTGGGTCAACTGGATGTCACA-3' | 2 |
| 403 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GCAAGCGATGGTGACTCTGTCTCCTACAGC-3' | 3 |
| 404 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TCTGTCCCAGATCCACTGCCACTGAACCTT-3' | 4 |
| 405 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GCAGCCACAGTTCGCTTCATCTGCACCTTG-3' | 5 |
| 406 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TTTCAACTGCTCATCAGATGGCGGGAAGAT-3' | 6 |
| 407 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AAGTTATTCAGCAGGCACACAACAGAGGCA-3' | 7 |
| 408 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGCGTTATCCACCTTCCACTGTACTTTGGC-3' | 8 |
| 409 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TAGGTGCTGTCCTTGCTGTCCTGCTCTGTG-3' | 9 |
| 410 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GTAGTCTGCTTTGCTCAGCGTCAGGGTGCT-3' | 10 |
| 411 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GATGGGTGACTTCGCAGGCGTAGACTTTGT-3' | 11 |
| 412 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CTCTCCCCTGTTGAAGCTCTTTGTGACGGG-3' | 12 |
| 413 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TGGAACTGAGGAGCAGGTGGGGGCACTTCT-3' | 13 |
| 414 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GAAAAAGGGTCAGAGGCCAAAGGATGGGAG-3' | 14 |
| 415 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AGATGAGCTGGAGGACCGCAATAGGGGTAG-3' | 15 |

| | | | |
|---|---|---|---|
| 416 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GCATAATTAAAGCCAAGGAGGAGGAGGGGG-3' | 16 |
| 501 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CCTGAGTGAGGAGGGTGAGGAGCAGCAGAG-3' | 17 |
| 502 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AGACCCAGACACGGAGGCAGGCTGAGTCAG-3' | 18 |
| 503 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TGTTGGTTCCAGTGCAGGAGATGGTGATCG-3' | 19 |
| 504 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TAAATCATGATTTTGGGGGCTTTGCCTGGG-3' | 20 |
| 505 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TGTTGCCAGACTTGGAGCCAGAGAAGCGAT-3' | 21 |
| 506 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AATAATCAGCCTCGTCCTCAGCCTGGAGCC-3' | 22 |
| 507 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GGTCCCTCCGCCGAAAACCACAGTGTAACT-3' | 23 |
| 508 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TTATGAGACACACCAGTGTGGCCTTGTTGG-3' | 24 |
| 509 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CTGCTCAGGCGTCAGGCTCAGATAGCTGCT-3' | 25 |
| 511 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ATGCGTGACCTGGCAGCTGTAGCTTCTGTG-3' | 26 |
| 512 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ATTCTGTAGGGGCCACTGTCTTCTCCACGG-3' | 27 |
| 513 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CCTCCCCTGGGATCCTGCAGCTCTAGTCTC-3' | 28 |
| 515 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TGAGGGTTTATTGAGTGCAGGGAGAAGGGC-3' | 29 |
| 221 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GGAGGTCAAAACAGCGTGGATGGCG-3' | 30 |
| 222 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GAGGCTGGATCGGTCCCGGTGTCTT-3' | 31 |
| 223 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AATCCGCGTTCCAATGCACCGTTCC-3' | 32 |
| 224 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TAAAAACTGCGGGCACTGGGGACGG-3' | 33 |
| 225 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ACCCGAGATTCGCGTGGAGATCCCA-3' | 34 |
| 226 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GAGCAAGGAGCTGCCGAGCGACCAT-3' | 35 |
| 227 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ACACTGGTGGTGGTGGGCATCGTGC-3' | 36 |
| 228 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TTCCAAATGCGTCAGCGGTGCAAGC-3' | 37 |
| 229 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AGCTGCCTGCATCTTCTTCTGCCGC-3' | 38 |

| | | |
|---|---|---|
| 238 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TCTCAGAGGATCGGCCCCCAGAATG-3' | 47 |
| 239 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCTCATCTGACTCCTCGGCGATGGC-3' | 48 |
| 240 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGGGTACAGGGGACTCTGGGGGTGA-3' | 49 |
| 241 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGGTGGGTGCTCTTGCCTCCAGAGG-3' | 50 |
| 100A2 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GACCTCGGGTCGGTAGCACCGCACT-3' | 51 |
| 100C2 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGAAGCCTCTCTTCTCCTCCCCCGG-3' | 52 |
| 100A1 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCACAGACACCGTCCTCACCACCCG-3' | 53 |
| 100B1 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGCTACAGCCACACACGTCTCCTCC-3' | 54 |
| 301 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGAGGCGGGCGGATCACCTGAGGTC-3' | 55 |
| 302 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 56 |
| 320 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TTTTTTTTTTTTTTTTTTTTTTTTT-3' | 57 |
| 301A | 5'-CTATTTTCTATTTTCTTTT CGAGGCGGGCGGATCACCTGAGGTC-3' | 116 |
| 302C | 5'-CTATTTTCTATTTTCTTTT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 117 |
| 302A4 | 5'-CTATTTTATACTTTATATTTCATATTTTATCT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 118 |
| 302A3/2 | 5'-CTATTTTATATTTATATTTCT CGGGAGGCGGAGGTTGCAGTGAGCC ACTATTTTATACTT-3 | 119 |
| 1002A32 | 5'-CTATTTTATACTTTATATTTCT GACCTCGGGTCGGTAGCACCGCAC TACTATTTTATACTT-3' | 120 |
| 301A2/2 | 5'-CTATTTTTCTT CGAGGCGGGCGGATCACCTGAGGTC TTCTTTTTATCTT-3 | 121 |
| 301A3/2 | 5'-CTATTTTATACTTTATATTTCT CGAGGCGGGCGGATCACCTGAGGTC ACTATTTTATACTT-3' | 122 |

**Table 2**

| Probe ID | Sequence | SEQ ID |
|---|---|---|
| | 5'-CTATTTTTCTATTTTTCTTTT | 123 |
| | 5'-CTATTTTATACTTTATATTTCATATTTTATCT | 124 |
| 330 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | 58 |
| | 5'-CTATTTTATACTTTATATTTCT.....ACTATTTTATACTT-3 | 125 |
| | 5'-CTATTTTTCTT.....TTCTTTTTATCTT-3 | 126 |

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

WE CLAIM:

1. An oligonucleotide label-domain comprising the sequence $(CTATTTT)_n$ and its complement $(AAAATAG)_n$ wherein "n" is at least 1.

2. The oligonucleotide label-domain of claim 1 detectably labeled with a reporter molecule, or a hapten molecule.

3. The oligonucleotide label-domain of claim 2 wherein the hapten is flurorescein linked to the N4 nitrogen of cytosine through an OBEA linker.

4. The oligonucleotide label-domain of claim 1 wherein the reporter molecule is a fluorophore.

5. The oligonucleotide label-domain of claim 1 wherein the fluorophore is present at a density of greater than 7 mole percent.

6. The oligonucleotide label-domain of claim 1 wherein the label-domain has the sequence $TC(TTTTATC)_n$ ( or its complementary formula).

7. The oligonucleotide label-domain of claim 1 wherein the sequence is SEQ ID NO: 58.

8. The oligonucleotide label-domain of claim 2 wherein at least 7 mole pecent of the cytosines are linked to a detectable moiety by an OBEA linker.

9. An oligonucleotide probe having at least two distinct functional domains, a first domain comprising the label-domain of claim 2, and a second domain comprising a gene-specific target sequence.

10. The oligonucleotide probe of claim 9 wherein the label-domain is located at the 5' end of the oligonucleotide probe, and the gene-specific target sequence being 3' to the label-domain.

11. The oligonucleotide probe of claim 9 wherein the label-domain is located at the 3' end of the oligonucleotide probe, and the gene-specific target sequence is 5' to the label-domain.

12. An oligonucleotide probe having three distinct functional domains, a first domain comprising the label-domain of claim 2, a second domain comprising a gene-specific target sequence, and a third domain comprising another label-domain, wherein said second domain is located between said first and third domains.

13. A probeset for detecting Kappa immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 401 through 416, inclusive.

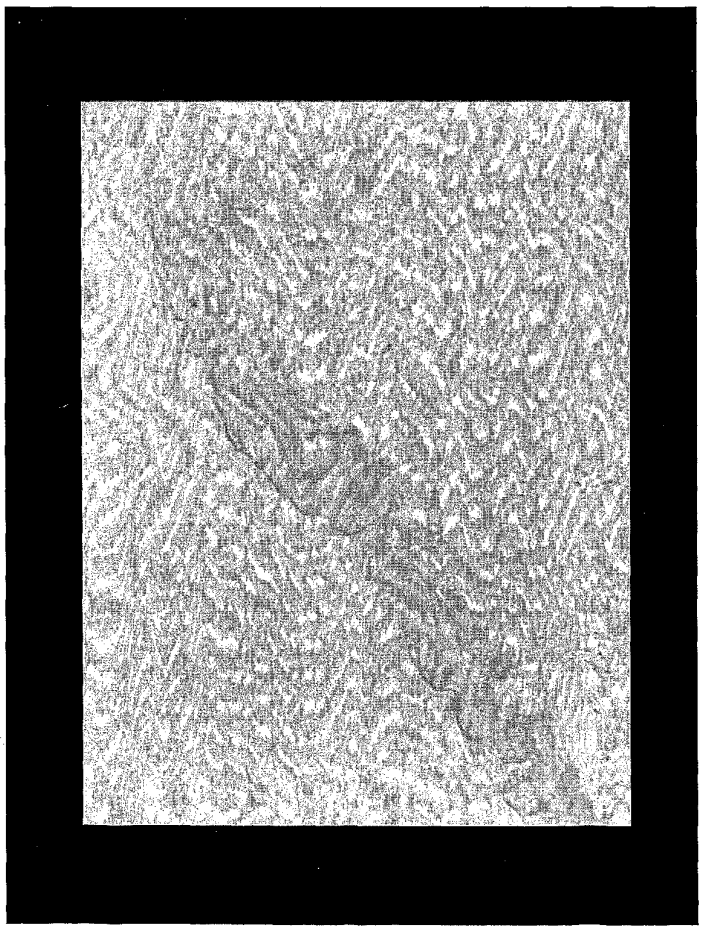
14. A probeset for detecting Lambda immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 501 through 509, 511-513, and 515.

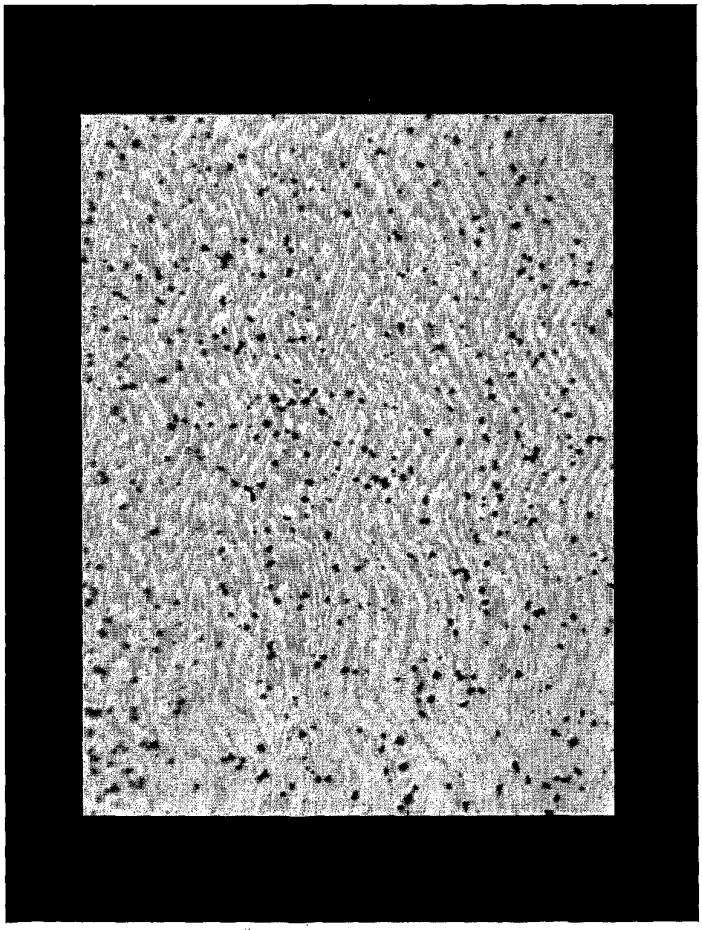
15. A probeset for detecting cytomegalovirus (CMV) immediate early RNA and /or corresponding mRNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 221 through 241

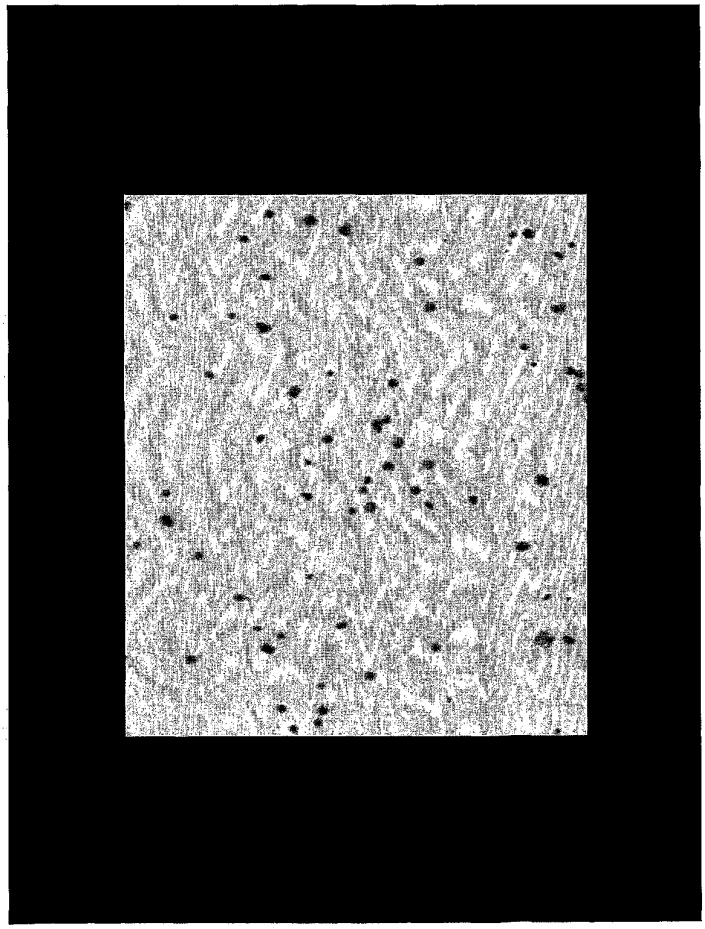
16. A probeset for detecting Epstein Barr virus (EBV) early RNA, RNA 1 and RNA 2, (EBER) wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 51 through 54.

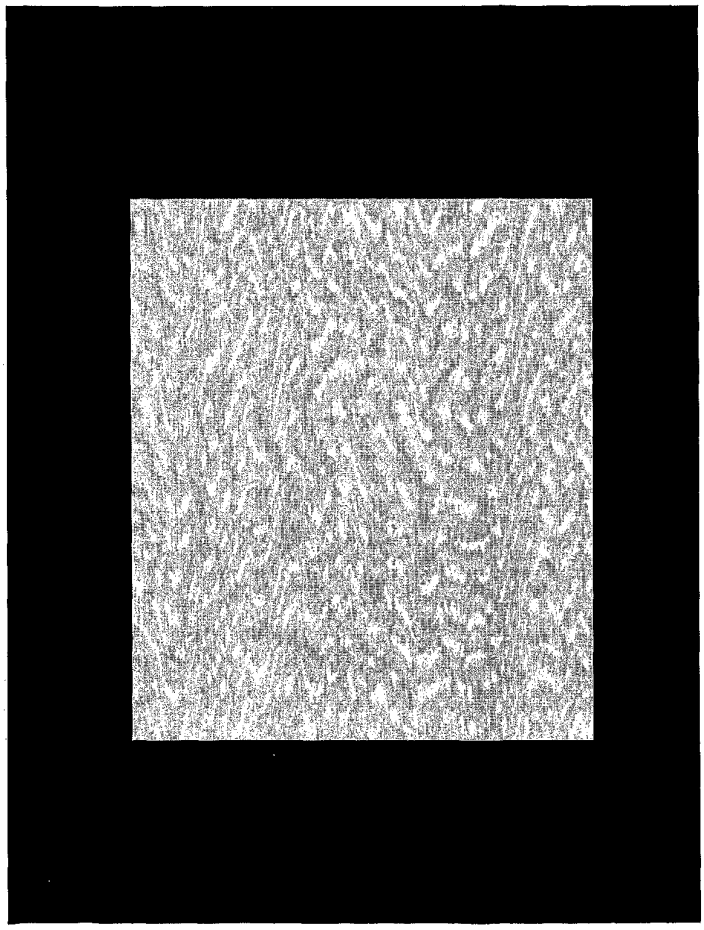
17. A probeset for detecting Human Alu repetitive sattelite genomic DNA sequences wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 301 and 302.

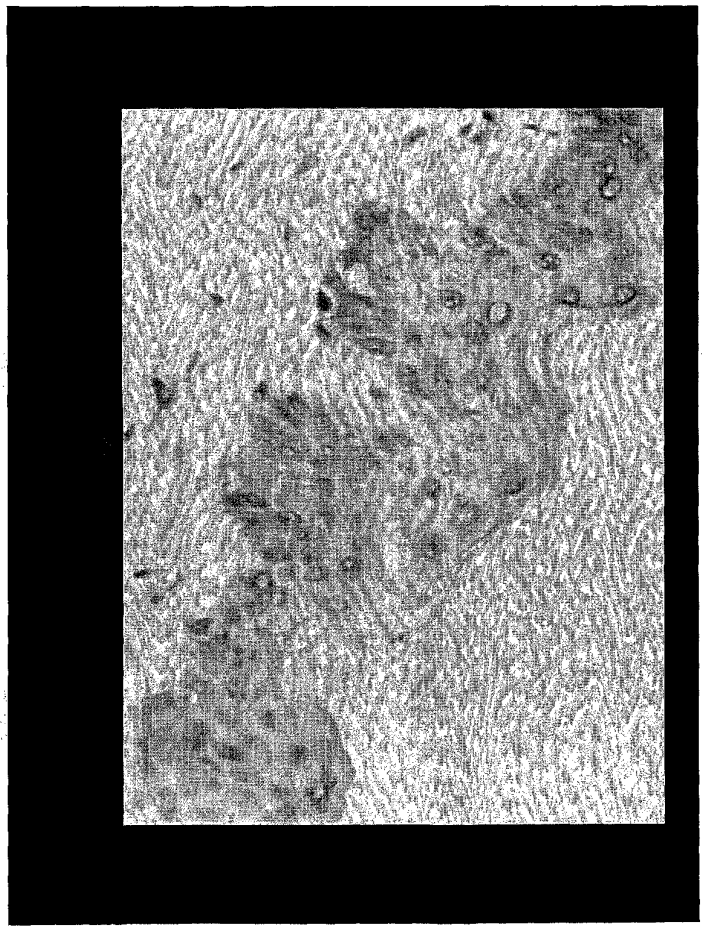
Oligonucleotide Probe Design
Common Signal Detection Region
Unique Target Detection Region
5'-
-3'
Alu301 5'-CTATTTTCTATTTTCTATTTTCT-CGAGGCGGGCGGGATCACCTGAGGTC-3'
Alu302 5'-CTATTTTCTATTTTCTATTTTCT-CGGGAGGCGGAGGTTGCAGTGAGCC-3'

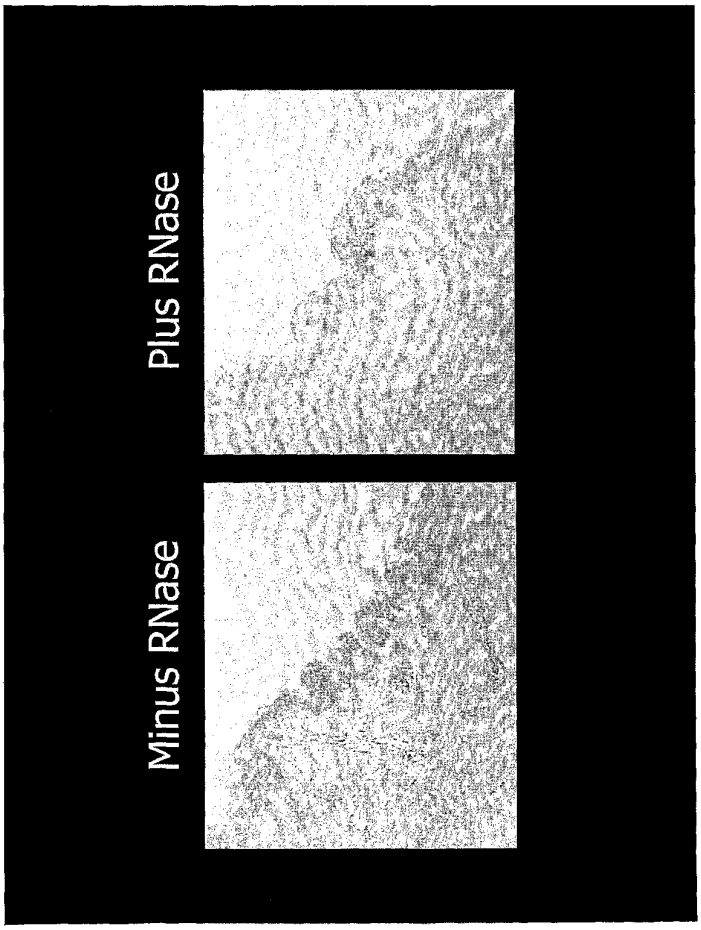








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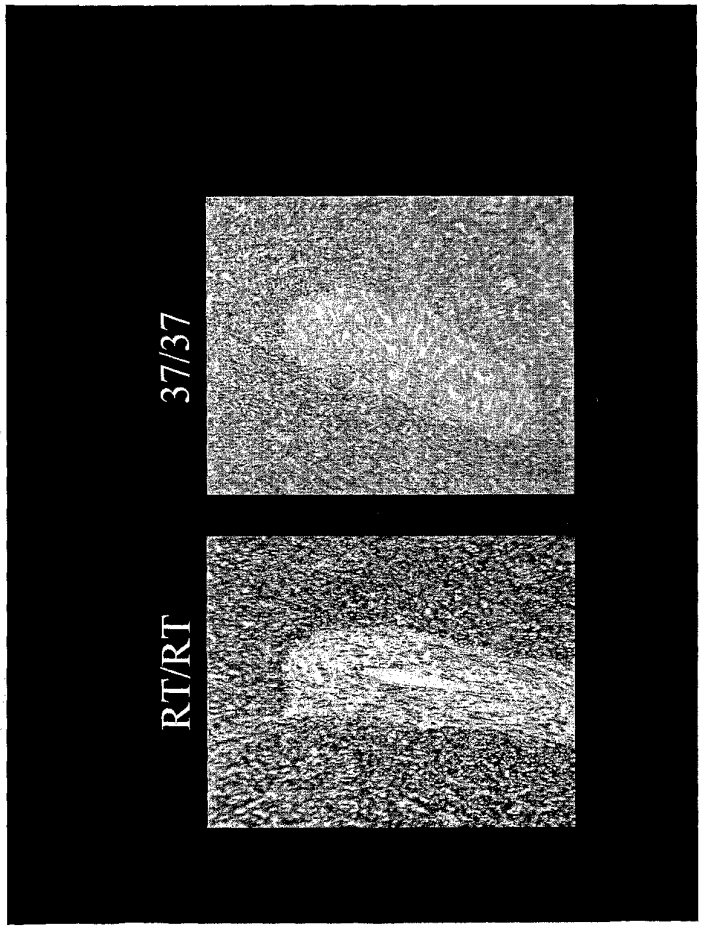
Minus RNase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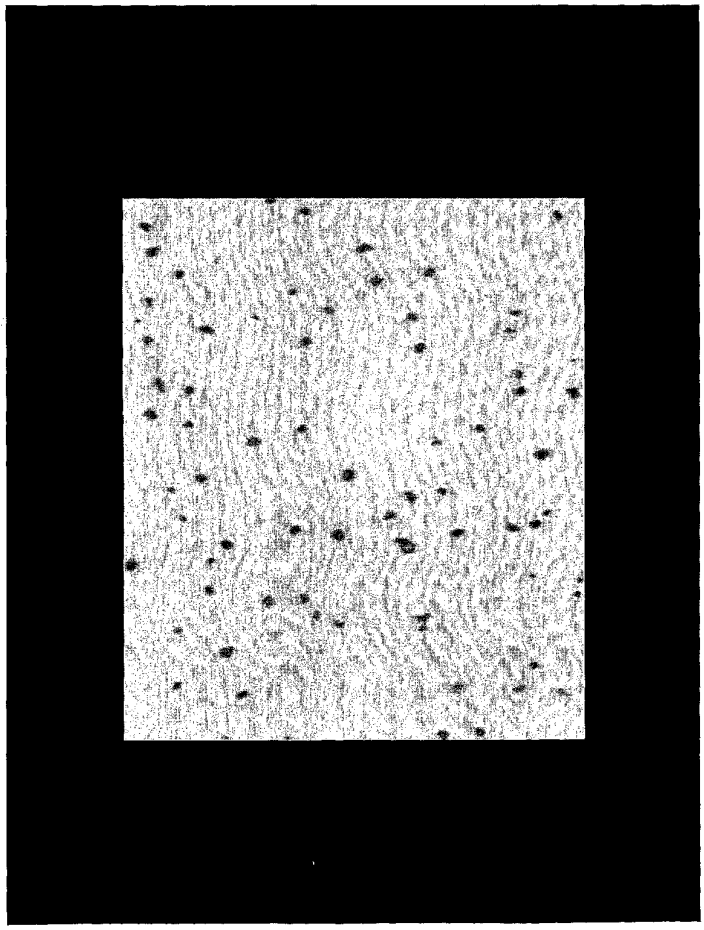

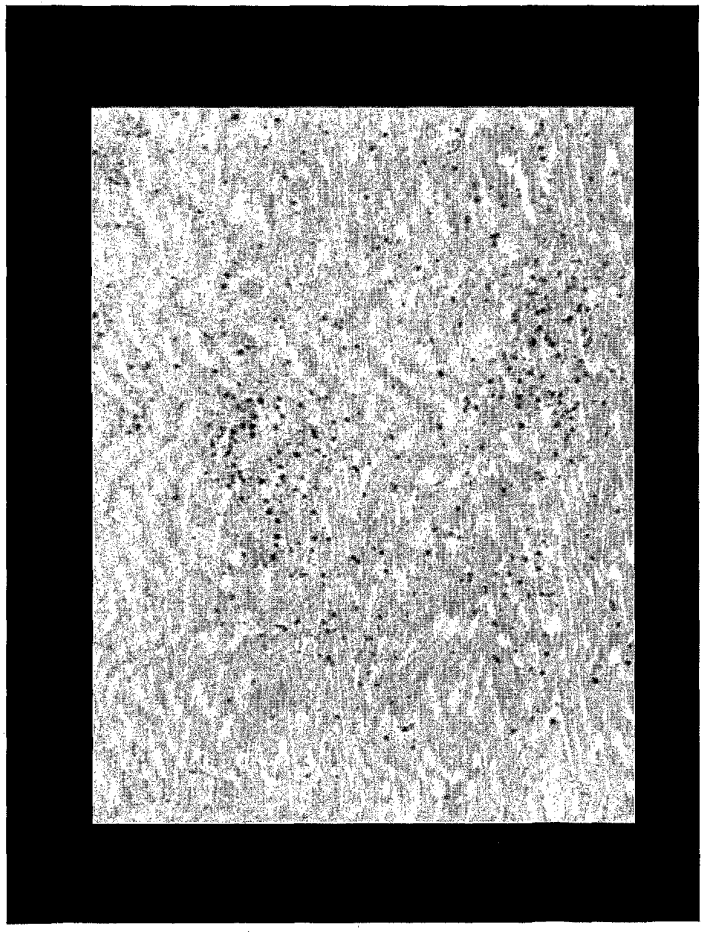

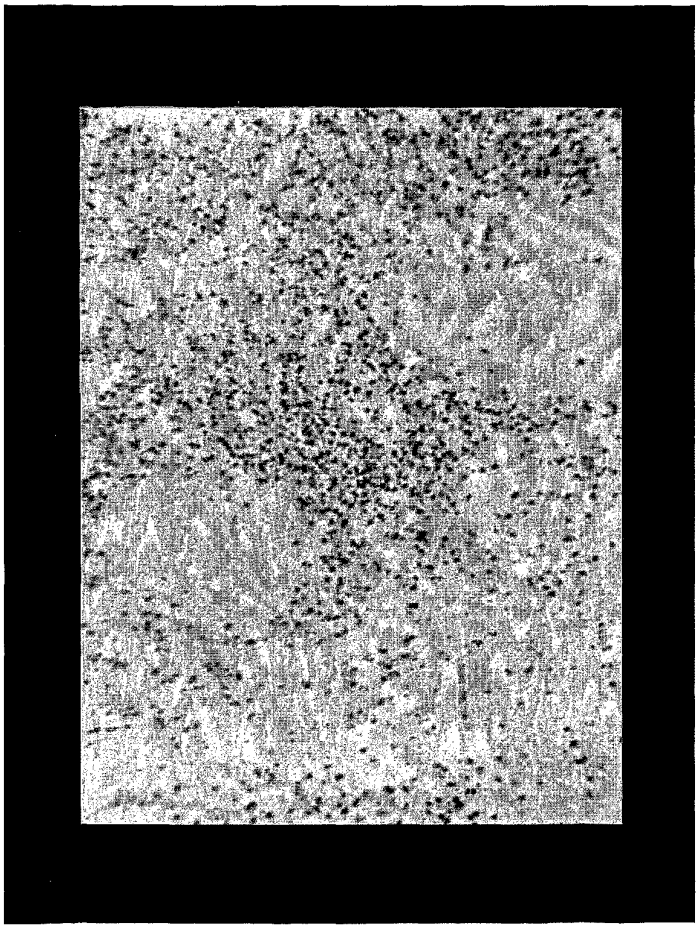

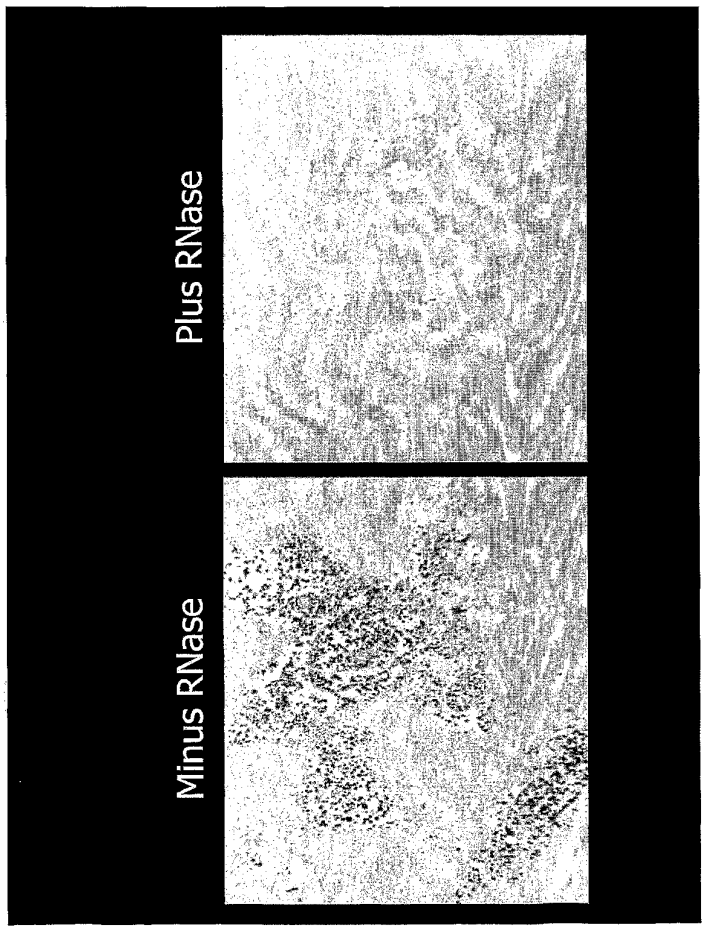
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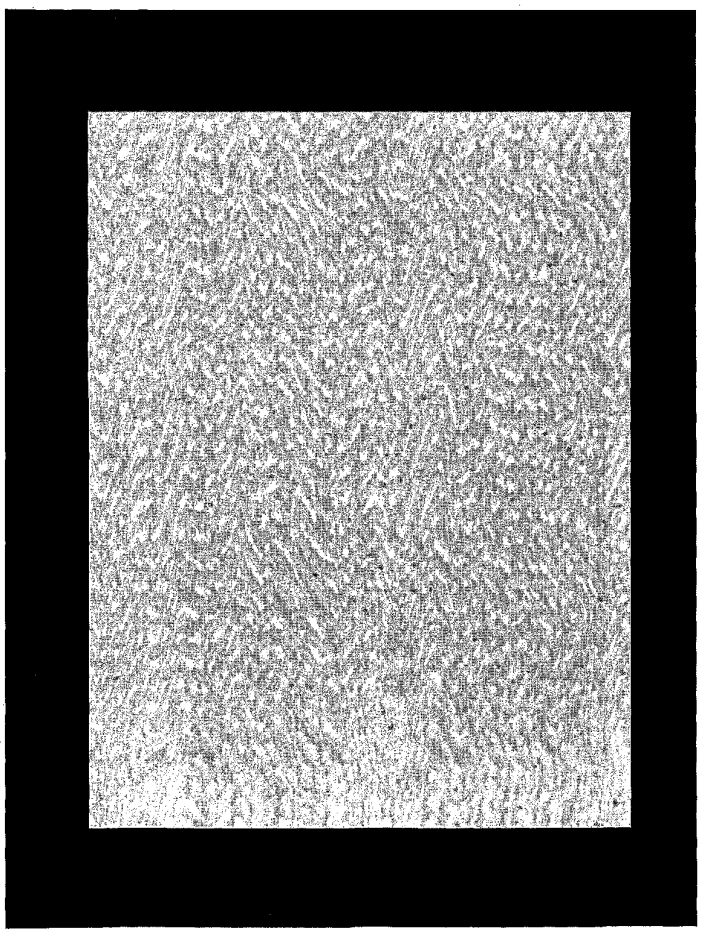
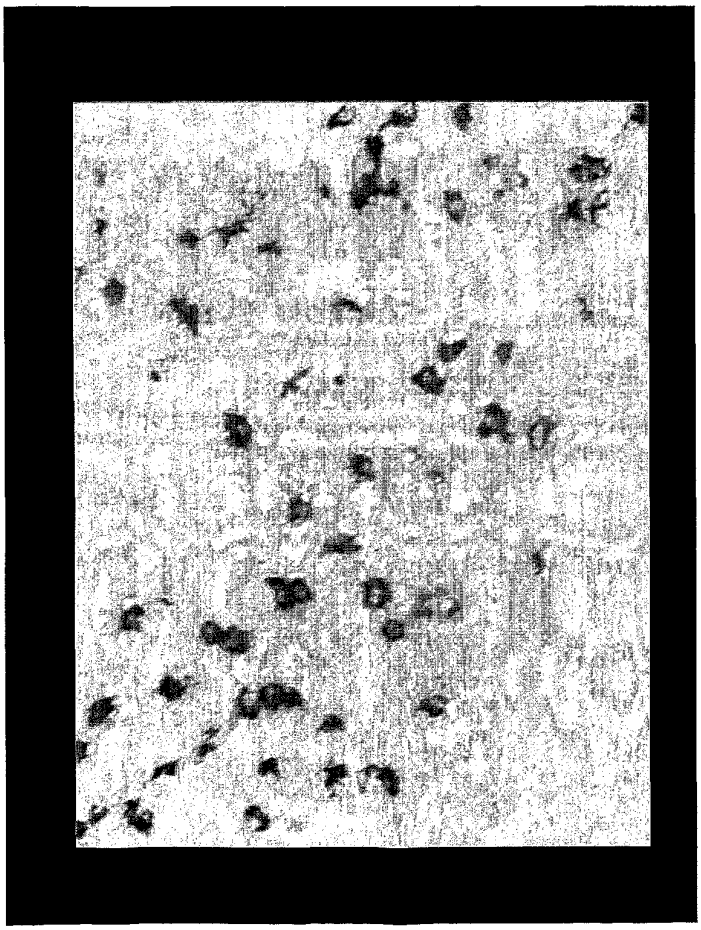
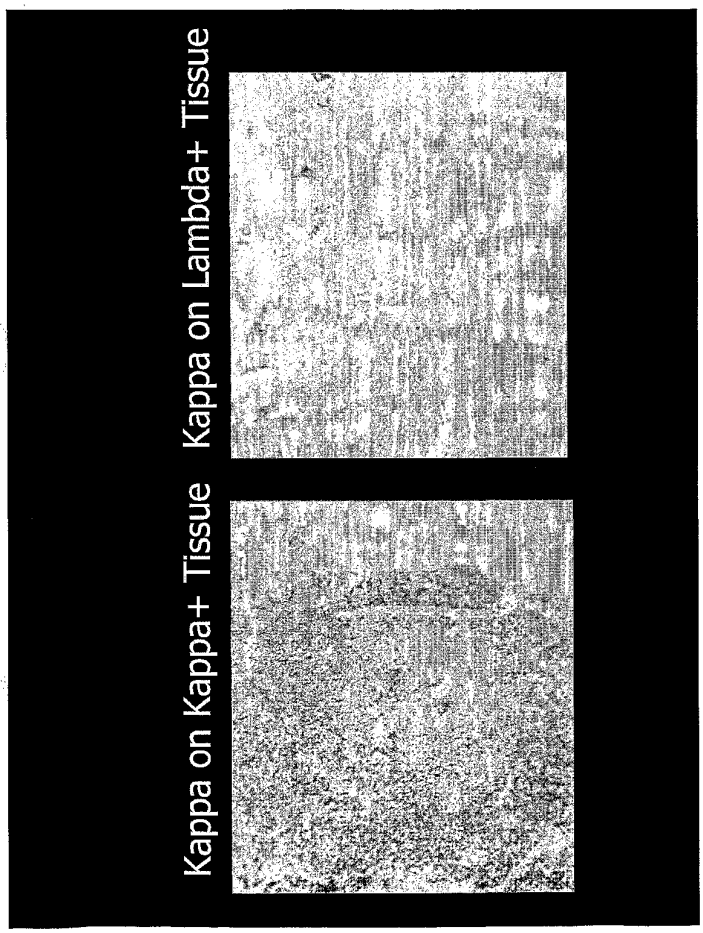
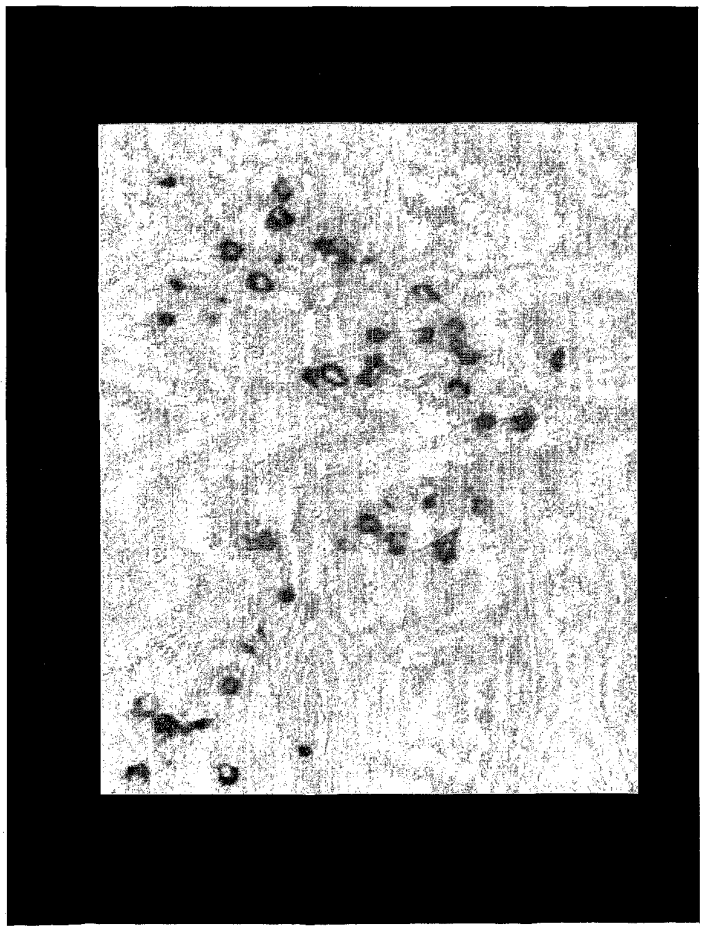
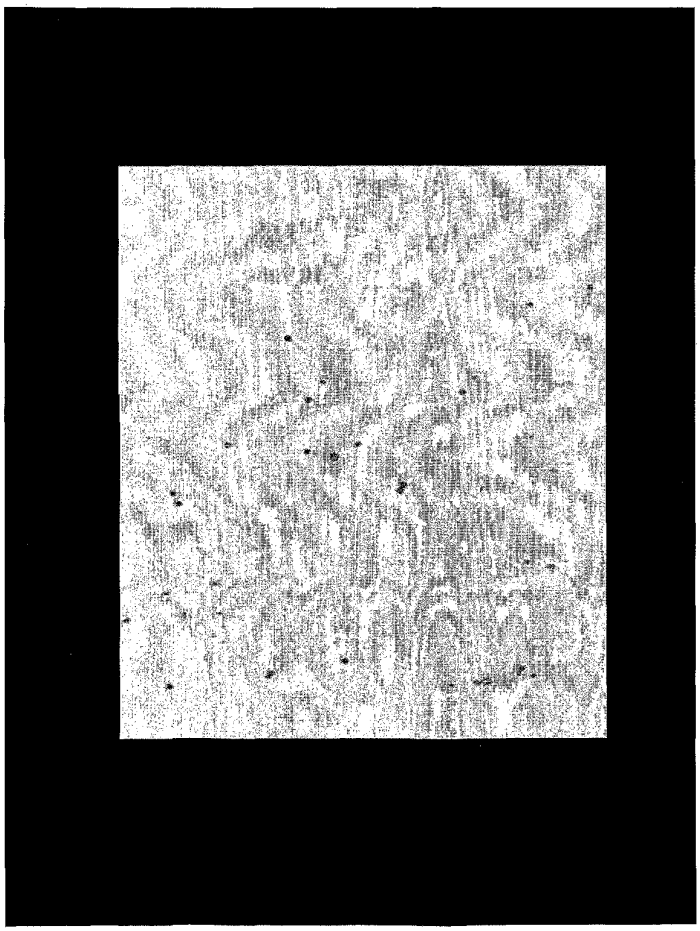
Minus RNase

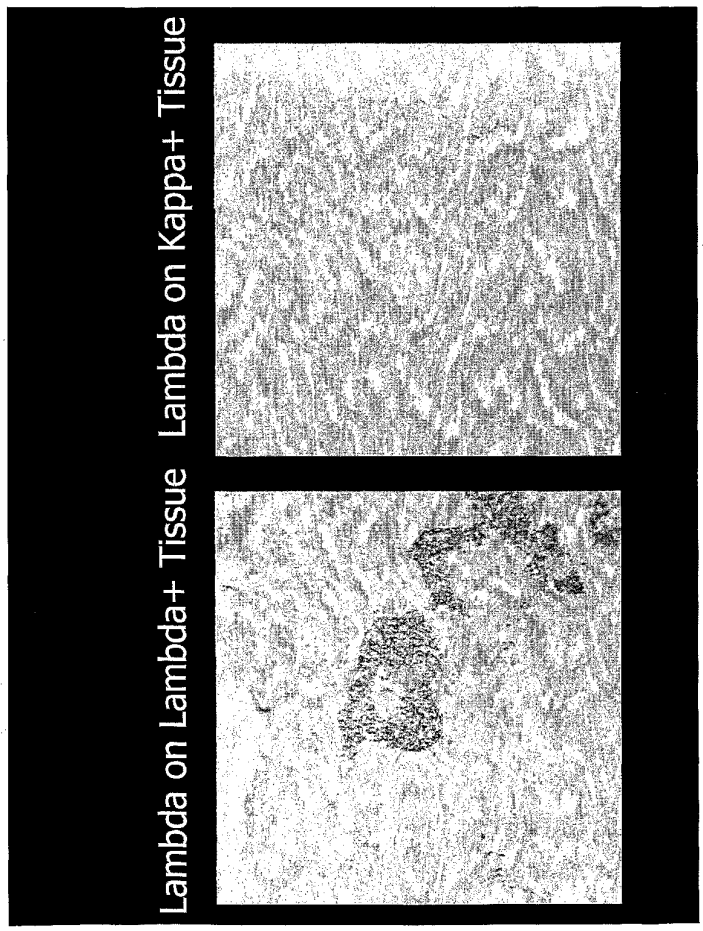


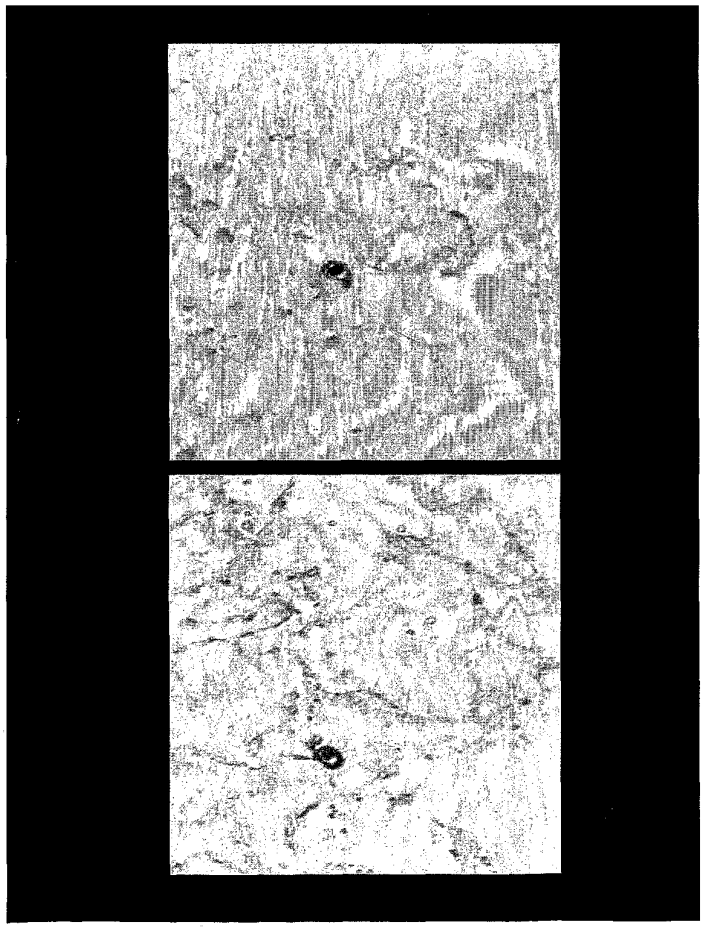


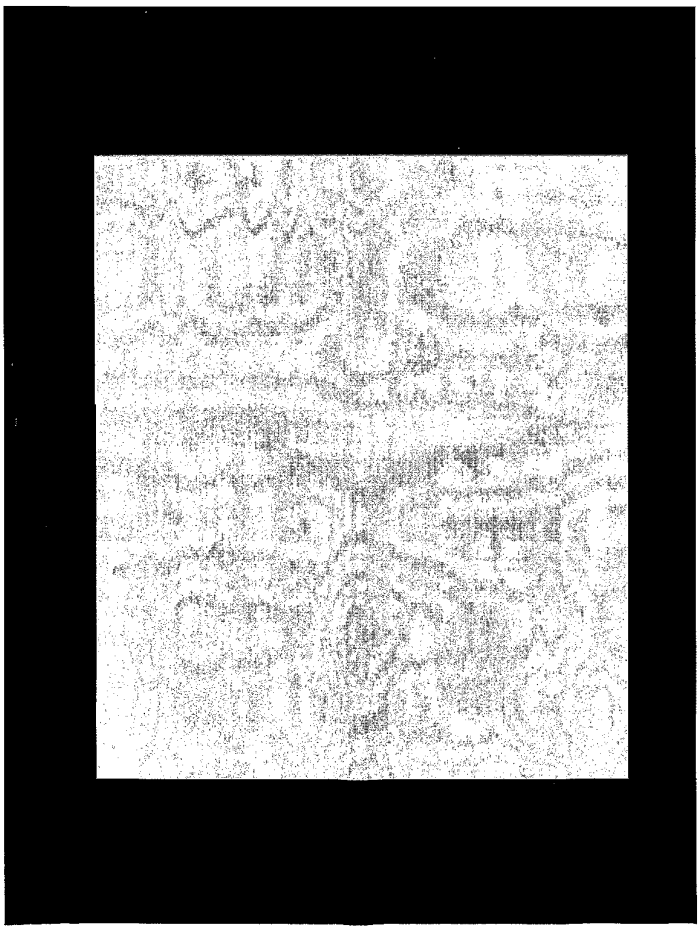

Kappa on Kappa+ Tissue
Kappa on Lambda+ Tissue

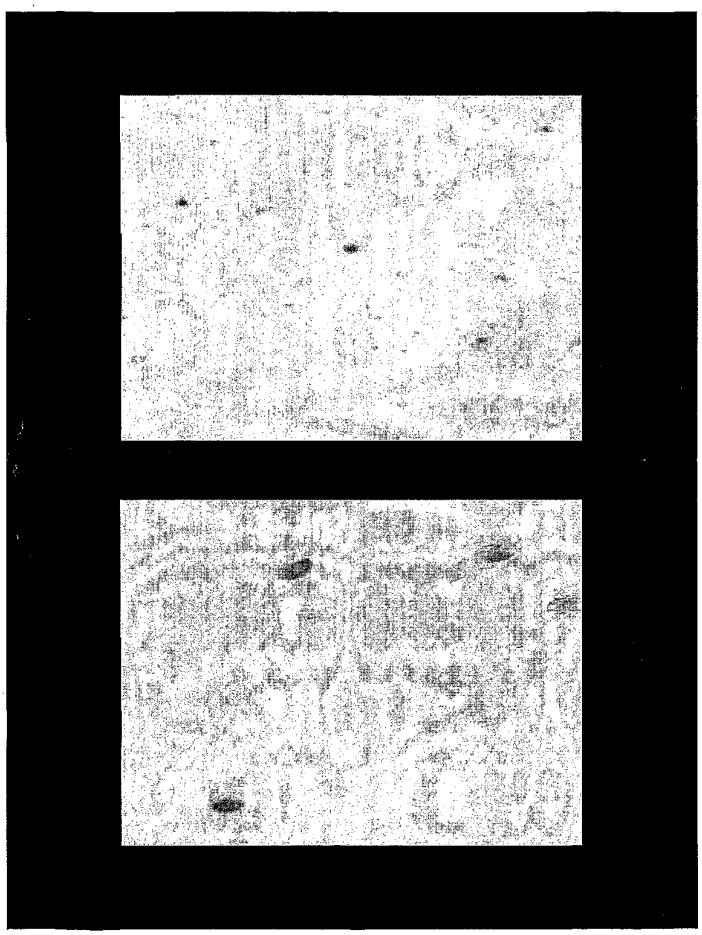





Lambda on Lambda+ Tissue
Lambda on Kappa+ Tissue







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<110> Connaughton, John

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Oligonucleotide probe

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Oligonucleotide probe

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Oligonucleotide probe

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Oligonucleotide probe

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Oligonucleotide probe

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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 16
ctattttcta ttttctattt tctattttct gcataattaa agccaaggag gaggaggggg 60

<210> 17
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 17
ctattttcta ttttctattt tctattttct cctgagtgag gagggtgagg agcagcagag 60

<210> 18
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 18
ctattttcta ttttctattt tctattttct agacccagac acggaggcag gctgagtcag 60

<210> 19
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 19
ctattttcta ttttctattt tctattttct tgttggttcc agtgcaggag atggtgatcg 60

<210> 20
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 20
ctattttcta ttttctattt tctattttct taaatcatga ttttgggggc tttgcctggg 60

<210> 21
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 21
ctattttcta ttttctattt tctattttct tgttgccaga cttggagcca gagaagcgat 60

<210> 22
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 22
ctattttcta ttttctattt tctattttct aataatcagc ctcgtcctca gcctggagcc 60

<210> 23

<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 23
ctattttcta ttttctattt tctattttct ggtccctccg ccgaaaacca cagtgtaact 60

<210> 24
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 24
ctattttcta ttttctattt tctattttct ttatgagaca caccagtgtg gccttgttgg 60

<210> 25
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 25
ctattttcta ttttctattt tctattttct ctgctcaggc gtcaggctca gatagctgct 60

<210> 26
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 26
ctattttcta ttttctattt tctattttct atgcgtgacc tggcagctgt agcttctgtg 60

<210> 27
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 27

ctattttcta ttttctattt tctattttct attctgtagg ggccactgtc ttctccacgg 60

<210> 28
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 28
ctattttcta ttttctattt tctattttct cctcccctgg gatcctgcag ctctagtctc 60

<210> 29
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 29
ctattttcta ttttctattt tctattttct tgagggttta ttgagtgcag ggagaagggc 60

<210> 30
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 30
ctattttcta ttttctattt tctattttct ggaggtcaaa acagcgtgga tggcg 55

<210> 31
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 31
ctattttcta ttttctattt tctattttct gaggctggat cggtcccggt gtctt 55

<210> 32
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:

Oligonucleotide probe

<400> 32
ctattttcta ttttctattt tctattttct aatccgcgtt ccaatgcacc gttcc 55

<210> 33
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 33
ctattttcta ttttctattt tctattttct taaaaactgc gggcactggg gacgg 55

<210> 34
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 34
ctattttcta ttttctattt tctattttct acccgagatt cgcgtggaga tccca 55

<210> 35
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 35
ctattttcta ttttctattt tctattttct gagcaaggag ctgccgagcg accat 55

<210> 36
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 36
ctattttcta ttttctattt tctattttct acactggtgg tggtgggcat cgtgc 55

<210> 37
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 37
ctattttcta ttttctattt tctattttct ttccaaatgc gtcagcggtg caagc 55

<210> 38
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 38
ctattttcta ttttctattt tctattttct agctgcctgc atcttcttct gccgc 55

<210> 39
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 39
ctattttcta ttttctattt tctattttct ccctccaccg ttaacagcac cgcaa 55

<210> 40
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 40
ctattttcta ttttctattt tctattttct ttggtcacgg gtgtctcggg cctaa 55

<210> 41
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 41
ctattttcta ttttctattt tctattttct tcggccaact ctggaaacag cgggt 55

<210> 42

<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 42
ctattttcta ttttctattt tctatttttct tcggggttct cgttgcaatc ctcgg 55

<210> 43
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 43
ctattttcta ttttctattt tctatttttct atctcgatgc cccgctcaca tgcaa 55

<210> 44
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 44
ctattttcta ttttctattt tctatttttct tgccgcacca tgtccactcg aacct 55

<210> 45
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 45
ctattttcta ttttctattt tctatttttct gttagcggcg cccttgctca catca 55

<210> 46
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 46

ctattttcta ttttctattt tctattttct tgcagatctc ctcaatgcgg cgctt 55

<210> 47
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 47
ctattttcta ttttctattt tctattttct tctcagagga tcggccccca gaatg 55

<210> 48
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 48
ctattttcta ttttctattt tctattttct cctcatctga ctcctcggcg atggc 55

<210> 49
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 49
ctattttcta ttttctattt tctattttct cgggtacagg ggactctggg ggtga 55

<210> 50
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 50
ctattttcta ttttctattt tctattttct gggtgggtgc tcttgcctcc agagg 55

<210> 51
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:

Oligonucleotide probe

<400> 51
ctattttcta ttttctattt tctattttct gacctcgggt cggtagcacc gcact 55

<210> 52
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 52
ctattttcta ttttctattt tctattttct ggaagcctct cttctcctcc cccgg 55

<210> 53
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 53
ctattttcta ttttctattt tctattttct ccacagacac cgtcctcacc acccg 55

<210> 54
<211> 56
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 54
ctattttcta ttttctattt tctattttct ggctacagcc acacacgtct cctccc 56

<210> 55
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 55
ctattttcta ttttctattt tctattttct cgaggcgggc ggatcacctg aggtc 55

<210> 56
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 56
ctattttcta ttttctattt tctattttct cgggaggcgg aggttgcagt gagcc 55

<210> 57
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 57
ctattttcta ttttctattt tctattttct tttttttttt tttttttttt tttttttttt 60

<210> 58
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 58
ctattttcta ttttctattt tctattttct 30

<210> 59
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 59
ccagagtagc aggagcccca ggagctgagc 30

<210> 60
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 60
ggatggagac tgggtcaact ggatgtcaca 30

<210> 61

<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 61
gcaagcgatg gtgactctgt ctcctacagc 30

<210> 62
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 62
tctgtcccag atccactgcc actgaacctt 30

<210> 63
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 63
gcagccacag ttcgcttcat ctgcaccttg 30

<210> 64
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 64
tttcaactgc tcatcagatg gcgggaagat 30

<210> 65
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 65

aagttattca gcaggcacac aacagaggca 30

<210> 66
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 66
ggcgttatcc accttccact gtactttggc 30

<210> 67
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 67
taggtgctgt ccttgctgtc ctgctctgtg 30

<210> 68
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 68
gtagtctgct ttgctcagcg tcagggtgct 30

<210> 69
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 69
gatgggtgac ttcgcaggcg tagactttgt 30

<210> 70
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:

Oligonucleotide probe

<400> 70
ctctcccctg ttgaagctct ttgtgacggg 30

<210> 71
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 71
tggaactgag gagcaggtgg gggcacttct 30

<210> 72
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 72
gaaaaagggt cagaggccaa aggatgggag 30

<210> 73
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 73
agatgagctg gaggaccgca ataggggtag 30

<210> 74
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 74
gcataattaa agccaaggag gaggaggggg 30

<210> 75
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 75
cctgagtgag gagggtgagg agcagcagag 30

<210> 76
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 76
agacccagac acggaggcag gctgagtcag 30

<210> 77
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 77
tgttggttcc agtgcaggag atggtgatcg 30

<210> 78
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 78
taaatcatga ttttgggggc tttgcctggg 30

<210> 79
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 79
tgttgccaga cttggagcca gagaagcgat 30

<210> 80

<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 80
aataatcagc ctcgtcctca gcctggagcc 30

<210> 81
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 81
ggtccctccg ccgaaaacca cagtgtaact 30

<210> 82
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 82
ttatgagaca caccagtgtg gccttgttgg 30

<210> 83
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 83
ctgctcaggc gtcaggctca gatagctgct 30

<210> 84
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 84

atgcgtgacc tggcagctgt agcttctgtg 30

<210> 85
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 85
attctgtagg ggccactgtc ttctccacgg 30

<210> 86
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 86
cctcccctgg gatcctgcag ctctagtctc 30

<210> 87
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 87
tgagggttta ttgagtgcag ggagaagggc 30

<210> 88
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 88
ggaggtcaaa acagcgtgga tggcg 25

<210> 89
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:

Oligonucleotide probe

<400> 89
gaggctggat cggtcccggt gtctt 25

<210> 90
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 90
aatccgcgtt ccaatgcacc gttcc 25

<210> 91
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 91
taaaaactgc gggcactggg gacgg 25

<210> 92
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 92
acccgagatt cgcgtggaga tccca 25

<210> 93
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 93
gagcaaggag ctgccgagcg accat 25

<210> 94
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 94
acactggtgg tggtgggcat cgtgc 25

<210> 95
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 95
ttccaaatgc gtcagcggtg caagc 25

<210> 96
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 96
agctgcctgc atcttcttct gccgc 25

<210> 97
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 97
ccctccaccg ttaacagcac cgcaa 25

<210> 98
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 98
ttggtcacgg gtgtctcggg cctaa 25

<210> 99

<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 99
tcggccaact ctggaaacag cgggt 25

<210> 100
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 100
tcggggttct cgttgcaatc ctcgg 25

<210> 101
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 101
atctcgatgc cccgctcaca tgcaa 25

<210> 102
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 102
tgccgcacca tgtccactcg aacct 25

<210> 103
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 103

gttagcggcg cccttgctca catca 25

<210> 104
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 104
tgcagatctc ctcaatgcgg cgctt 25

<210> 105
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 105
tctcagagga tcggccccca gaatg 25

<210> 106
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 106
cctcatctga ctcctcggcg atggc 25

<210> 107
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 107
cgggtacagg ggactctggg ggtga 25

<210> 108
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:

Oligonucleotide probe

<400> 108
gggtgggtgc tcttgcctcc agagg 25

<210> 109
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 109
gacctcgggt cggtagcacc gcact 25

<210> 110
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 110
ggaagcctct cttctcctcc cccgg 25

<210> 111
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 111
ccacagacac cgtcctcacc acccg 25

<210> 112
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 112
ggctacagcc acacacgtct cctccc 26

<210> 113
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 113
cgaggcgggc ggatcacctg aggtc 25

<210> 114
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 114
cgggaggcgg aggttgcagt gagcc 25

<210> 115
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 115
ttttttttt ttttttttt ttttttttt 30

<210> 116
<211>
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 116
CTATTTTTCTATTTTTCTTTT CGAGGCGGGCGGATCACCTGAGGTC 46

<210> 117
<211>
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 117
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<210> 118

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【国際公開パンフレット（コレクトバージョン）】

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 March 2002 (21.03.2002)

PCT

(10) International Publication Number
WO 02/022874 A2

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number: PCT/US01/28014

(22) International Filing Date:
6 September 2001 (06.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/233,177 15 September 2000 (15.09.2000) US

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(81) Designated States *(national)*: AU, CA, JP, MX, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

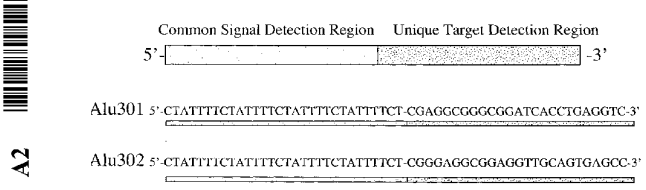
**Published:**
— *without international search report and to be republished upon receipt of that report*

(48) Date of publication of this corrected version:
3 April 2003

(15) Information about Correction:
see PCT Gazette No. 14/2003 of 3 April 2003, Section II

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: OLIGONUCLEOTIDE SEQUENCE FORMULA FOR LABELLING OLIGONUCLEOTIDE PROBE AND PROTEINS FOR IN-SITU ANALYSIS




(57) Abstract: The present invention provides oligonucleotide probes and oligonucleotide probe collections and protein labeling for detecting or localizing a plurality nucleic acid target genes or antigens within a cell or tissue sample. Specifically, the provides collections of oligonucleotide probe for use in *in situ* hybridization analyses in which each probe has a label-domain with the sequence formulas of $(CTATTTT)_n$, $(AAAATAG)_n$ or $(TTTTATC)_n$ or $(GATAAAA)_n$ in which all cases "n" would equal 1 or greater. The present invention provides collections or "cocktails" of oligonucleotide probes for detecting or localizing specific nucleic acid target genes within a cell or tissue sample. The cocktails are useful for detecting the following: the Kappa gene (SEQ ID NOS: 1-16 inclusive); the Lamba gene (SEQ ID NOS: 501-509, 511-513, and 515); the CMV (cytomegalovirus) gene (SEQ ID NOS: 221-241 inclusive); EBER (Epstein-Barr RNA) gene (SEQ IN NOS: 51-54 inclusive): Alu (SEQ IN NOS: 55-56); PolyA (SEQ ID NO: 57); and the detection tail (SEQ ID NO: 330).

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# OLIGONUCLEOTIDE SEQUENCE FORMULA FOR LABELING OLIGONUCLEOTIDE PROBES AND PROTEINS FOR *IN SITU* ANALYSIS

## BACKGROUND OF THE INVENTION

### 1. Field of the Invention

This invention relates to oligonucleotide probes and collections of oligonucleotide probes for detecting or localizing nucleic acid genes targets witin a cell or tissue sample. In particular, the invention relates to collections of oligoprobes.

### 2. Background of the Invention

*In situ* analysis includes in situ hybridization and immunohistochemistry. *In situ* hybridization (ISH) employs labeled DNA or RNA probe molecules that are anti-sense to a target gene sequence or transcript to detect or localize targeted nucleic acid target genes within a cell or tissue sample. ISH has proven to be a useful tool in a number of biomedical fields, including developmental biology, cell biology, and molecular biology. ISH has been used, for example, to diagnose genetic disorders, map genes, study gene expression, and localize sites of target gene expression.

Typically, ISH is performed by exposing a cell or tissue sample immobilized on a glass slide to a labeled nucleic acid probe which is capable of specifically hybridizing to a given target gene in the cell or tissue sample (*In Situ Hybridization: Medical Applications* (G. R. Coulton and J. de Belleroche, eds., Kluwer Academic Publishers, 1992); *In Situ Hybridization: In Neurobiology; Advances in Methodology* (J. H. Eberwine, K. L. Valentino, and J. D. Barchas, eds., Oxford University Press, 1994); *In Situ Hybridization: A Practical Approach* (D. G. Wilkinson, ed., Oxford University Press, 1992)). The hybridization of labeled probe molecules to nucleic acids in the cell or tissue sample can then be detected using, for example, radioactive-based direct detection methods, fluorescence-based direct detection methods, or indirect detection methods based on the binding of a fluorescence-labeled protein binding to a hapten such as BrdU, digoxigenin-labeled or biotin-labeled nucleotides incoporated into probes. Hapten-based methods have been further extended to include those molecules to be bonded by binding protein-enzyme conjugates such as antibody-enzyme-conjugates and colorimetric based detection chemistry. In addition, several target genes can be simulanteously analyzed by exposing a cell or tissue sample to a plurality of nucleic acid probes that have been

labeled with a plurality of different nucleic acid tags. For example, a plurality of nucleic acid probes can be labeled with a plurality of fluorescent compounds having different emission wavelengths, thereby permitting simultaneous multicolored analysis to be performed in a single step on a single target cell or tissue sample.

A significant problem associated with incorporation of labeled nucleotides into oligonucleotide probes is that the conjugation moieties that are attached to the nucleotide usually interfere with the formation of Watson-Crick base pairing, thus negatively affecting the hybridization of the probe to its target. The has been seen with use of label attached via N4-substitued cytosine nucleotides, because of steric hinderance and the expected shift to the less reactive state of a secondary amine (as seen with N4 labled cytosine), as compared to the natural G-C bond formed with an unsubstituted cytosine (a primary amine). Any small change or interference with G-C bonding in a small oligonucleotide (25 to 50 bases) can reduce the ability of these oligos to hybridize with the intended targeted sequence.

There remains a need in the art to develop suitable probes designs for incorporating labeled nucleotides in oligonucleotide probes. We demonstrate that a few artificial seqeunces are viable alternatives for probe labeling and also work both singly and in complex oligonucleotide probe mixtures for detecting or localizing nucleic acid target genes within a cell or tissue sample. The development of such generic seqeunces and labeling strategy for probe collections has wide application in the medical, genetic, and molecular biological arts.

This interference due to labeling chemistry and hybridization stringency and kinetics is solved herein by designing the oligo to have at least two distinct functional domains, one domain or sequence to be gene specific and involved in the base pair formation, and the second domain to be an artificial, non-specific sequence (in reference to the sample's genome) comprised of spacing nucleotides and the labeled nucleotide. These elements are positioned so that these label-nucleotides are more accessible as haptens for binding proteins (immunoglobulin or avidin(s)) and thus do not interfere with Watson-Crick base pairing in the gene-specific domain.

## SUMMARY OF THE INVENTION

The present invention provides a novel strategy to incorporate label into oligonucleotide probes and labeled oligonucleotide probe collections for detecting or localizing nucleic acid target genes within a cell or tissue sample. In particular, the invention relates to non-gene-specific sequences using sequence formulas for making repetitive polymers of such sequences which can be incorporated into collections of oligonucleotide probes for use in *in situ* hybridization analyses. In addition, using labeled synthetic oligonucleotide polymers, based on sequence formulas, when conjugated to binding proteins, i.e. immunglobulins, is a very effective and controlled process for labeling such proteins used in immunohistochemical analysis. The present invention provides collections or "cocktails" of oligonucleotide probes for detecting or localizing specific nucleic acid target genes within a cell or tissue sample. The cocktails are useful for detecting the following: the Kappa gene (SEQ ID NOS: 1-16 inclusive); the Lamba gene (SEQ ID NOS: 501-509, 511-513, and 515); the CMV (cytomegalovirus) gene (SEQ ID NOS: 221-241 inclusive); EBER (Epstein-Barr early RNA) gene (SEQ ID NOS: 51-54 inclusive); Alu (SEQ ID NOS: 55-56); PolyA (SEQ ID NO: 57); and the detection tail (SEQ ID NO:330).

The invention is directed to an oligonucleotide label-domain comprising the sequence $(CTATTTT)_n$ and its complement $(AAAATAG)_n$ wherein "n" is at least 1.

The invention is also directed to an oligonucleotide probe having at least two distinct functional domains, a first domain comprising the label-domain of claim 2, and a second domain comprising a gene-specific target sequence.

The invention is also directed to a probeset for detecting Kappa immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 401 through 416, inclusive.

The invention is also directed to a probeset for detecting Lambda immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 501 through 509, 511-513, and 515.

The invention is also directed to a probeset for detecting cytomegalovirus (CMV) immediate early RNA and /or corresponding mRNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 221 through 241.

The invention is also directed to a probeset for detecting Epstein Barr virus (EBV) early RNA, RNA 1 and RNA 2, (EBER) wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 51 through 54.

The invention is also directed to a probeset for detecting Human Alu repetitive satellite genomic DNA sequences wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 301 and 302.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a generic probe structure of of the two-domain probe design. This is the oligonucleotide design used for the probes in the gene specific cocktails described in the following examples. Each probe is composed of two domains: a 5' labeling domain and a 3' target gene target gene-specific domain. The labeling domain consists of this specific sequence (CTATTTT)n, wherein each cytosine may be labeled with a fluorophore or a cytosine-hapten conjugate, the hapten being fluorescein in this embodiment. This illustration specifically shows nucleic acid sequences for the 301 (SEQ ID NO: 55) and 302 (SEQ ID NO: 56) probes, each of which possesses target gene gene-specific domains corresponding to human repetitive Alu sequences and labeling domains having a fluorescein hapten.

Figure 2 illustrates the results obtained for *in situ* hybridization (ISH) analysis of human skin tissue using a probe comprising the labeling domain (330 probe; SEQ ID NO: 58). The absence of a detectable signal indicates that the sequence formula, (CTATTTT)n, of the labeling domain common to the oligonucleotides used in these ISH examples is non-specific, and non-reactive in its ability to form Watson-Crick base pairing with human nucleic acid sequences because it does not hybridize.

Figure 3 illustrates the results obtained for ISH analysis of human skin tissue using a probe comprising the labeling domain and a poly d(T) target gene-specific domain (320 probe; SEQ ID NO: 57). The presence of a detectable signal localized to the cytoplasm indicates that this probe is capable of specifically hybridizing to polyadenylated region of messenger RNA.

Figures 4A-4B illustrate the results obtained for ISH analysis of human skin tissue using the 320 probe, wherein the tissue sample was not treated with ribonuclease A prior

to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The decrease in detectable signal in (B) indicates that this probe specifically hybridizes to polyadenylated region common to messenger RNA. .

Figures 5A-5B illustrate the results obtained for ISH analysis of human spleen tissue using the 320 probe, wherein the hybridization and stringency wash were performed at room temperature (A), or at 37°C (B). This result illustrates that the intenstity of color is related to the stringency of hybridization conditions, with the more intense color indicating less stringent conditons.

Figure 6 illustrates the results obtained for ISH analysis of the human Raji cell line using the 320 probe. This shows that this probe design also is functional with embedded cell lines as well as embedded tissue.

Figure 7 illustrates the results obtained for ISH analysis of the human Raji cell line using a probe collection consisting of the 301 and 302 probes.

Figure 8 illustrates the results obtained for ISH analysis of the human HT cell line using a probe collection consisting of the 301 and 302 probes.

Figure 9 illustrates the results obtained for ISH analysis of a rat cell line using a probe collection consisting of the 301 and 302 probes. The absence of a detectable signal indicates that this probe collection is specific for human nucleic acid sequences.

Figure 10 illustrates the results obtained for ISH analysis of an Epstein-Barr virus (EBV)-negative human HT cell line using a probe possessing a target gene-specific domain corresponding to EBV EBER nuclear RNA [SEQ ID NO: 51 through SEQ ID NO: 54].

Figure 11 illustrates the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figure 12 illustrates the results obtained for ISH analysis of human tonsil tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figures 13A-13B illustrate the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through

SEQ ID NO:54 , wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The decrease in detectable signal in (B) indicates that this probe specifically hybridizes to human EBER 1 and EBER 2 nuclear RNA.

Figure 14 illustrates the results obtained for ISH analysis of kappa light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain mRNA [ SEQ ID NO:15 ].

Figure 15 illustrates the results obtained for ISH analysis of lymphoma tissues using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin kappa light chain mRNA [SEQ ID NOS: 2 - 4, SEQ ID NOS:7-12, SEQ ID NOS: 14, 15]. The lymphoma tissue in (A) over expresses the kappa light chain and the tissue in (B) over expresses the lambda light chain. The absence of a detectable signal in (B) indicates that the kappa light chain probe collection is specific to kappa light chain mRNA.

Figure 16 illustrates the results obtained for ISH analysis of lambda light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain variable region mRNA [ SEQ ID NOS:19 through 29].

Figure 17 illustrates the results obtained for ISH analysis of a lambda light chain-positive human RPMI 8226 cell line using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [ SEQ ID NOS:19 through 29].

Figures 18A-18B illustrate the results obtained for ISH analysis of human lymphoma tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [ SEQ ID NOS:19 through 29]. The tissue in (A) over expresses the lambda light chain and the tissue in (B) over expresses the kappa light chain. The absence of a detectable signal in (B) indicates that the lambda light chain probe collection is specific to human lambda light chain mRNA.

Figure 19 illustrates the results obtained for ISH analysis of cytomeglovirus (CMV)-positive human lung tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [

SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50] [ CMV infected cell ].

Figure 20 illustrates the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has not been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early mRNA [ SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50].

Figures 21A-21B illustrate the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50. The tissue in (A) is shown at a magnification of 40X and the tissue in (B) is shown at a magnification of 20X.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides oligonucleotide probes and oligonucleotide probe collections for detecting or localizing nucleic acid target genes within a cell or tissue sample. In particular, the invention relates to collections of oligonucleotide probes for use in *in situ* hybridization analyses.

More specifically, this invention relates to the use of specific sequence formulas for nucleotide polymers or label-domains to attach a detectable moity ( a label) to oligonucleotide probes or proteins. The specific utility of these seqeuces or derivatives thereof, is the inert or non-reactive characteristic that does not hybridize to human DNA or RNA at a detectable level under standard stringency of hybridization conditions. These label-domains or polymers were demonstrated to be useful generic sequences for incorporation into oligonucleotide probes for detecting gene-specific sequences within cells or tissue samples in in situ hybridizaiton analyses. Additionally, this inert set of sequences are useful for attaching a label to immunoglobulins or other proteins for detecting haptens and antigens in immunohistochemical analyses.

As used herein, the terms "probe" or "oligonucleotide probe" refers to a nucleic acid molecule used to detect a complementary nucleic acid target gene.

As used herein, the term "hybridization" refers to the process whereby complementary nucleic acid sequences join to form a double-stranded nucleic acid

molecule. By labeling the target nucleic acid molecule with, for example, a radioactive or fluorescent tag, interactions between probe and target genes can be detected.

The oligonucleotide probes and oligonucleotide probes of the collections of the present invention are synthesized using conventional methods. *See e.g., Methods in Molecular Biology, Vol 20: Protocols for Oligonucleotides and Analogs* 165-89 (S. Agrawal, ed., 1993); *Oligonucleotides and Analogues: A Practical Approach* 87-108 (F. Eckstein, ed., 1991).

In a preferred embodiment of the present invention, oligonucleotide probes possess two distinct domains: a 5' (or labeling) domain and a 3' (or gene-specific target) domain (*See* Figure 1A). In more preferred embodiments of the present invention, the oligonucleotide probe possesses a labeling domain which consists of the sequence (CTATTTT)n. Other embodiments are also demonstrated herein, including a triple-domain embodiment having two terminal labeling domains, and a central gene-specific target domain. Specifically, SEQ ID NOS: 125-126 depict this labeling scheme. Yet a further preferred embodiment of a labeling domain is $TC(TTTTATC)_n$ or its complement. This sequence is predicted to be as unique as the (CTATTTT)n label-domain. The oligonucleotide probes of the present invention are labeled so that hybridization between said probes and target nucleic acids in a particular cell or tissue can be detected. Labels that are acceptable for use in *in situ* hybridization (ISH) analysis are known to those with skill in the art. Such labels permit interactions between probe and target genes to be detected using, for example, radioactive-based direct detection methods, fluorescence-based direct detection methods, digoxigenin-labeled or biotin-labeled probes coupled with fluorescence-based detection methods, or digoxigenin-labeled or biotin-labeled probes coupled with antibody-enzyme-based detection methods. In perferred embodiments of the present invention, oligonucleotide probes are labeled with fluorescein. In more preferred embodiments of the present invention, the oligonucleotide probe possesses a labeling domain which consists of the sequence (CTATTTT)n, wherein the cytosine nucleotides may be labeled with a flurophore for direct detection, or a hapten for indirect detection. In either, the fluorescein-cytosine nucleotide conjugate and the fluorescein molecule is linked at the N4 position of cytosine through an OBEA linkage (*See* Mishra *et al.*, U.S. Patent No. 5,684,142, which is incorporated herein by reference). In a preferred embodiment, the density of fluorphore attached to the label-domain is at least 7 mole percent, preferably at least 10 mole

percent, and most preferably at least 16 mole percent, when measured against the label-domain solely. For example, if probe 401 is considered (a 2-domain probe) it comprises a label-domain of 30 bases including a 3' terminal CT wherein the C is also labeled, the mole percent is 5/30 = 16.7 mole percent label. In the overall probe, the mole percent is 8.3.

In some embodiments of the present invention, several target genes are simulanteously analyzed by exposing a cell or tissue sample to a plurality of nucleic acid probes that have been labeled with a plurality of different nucleic acid tags. For example, a plurality of nucleic acid probes can be labeled with a plurality of fluorescent compounds having different emission wavelengths, thereby permitting simultaneous multicolored analysis to be performed in a single step on a single target cell or tissue sample.

The oligonucleotide probes and oligonucleotide probe collections of the present invention may be used in ISH analysis to detect or localize nucleic acid target genes within a cell or tissue sample. ISH may be performed as described, for example, in *In Situ Hybridization: Medical Applications* (G. R. Coulton and J. de Belleroche, eds., Kluwer Academic Publishers, 1992); *In Situ Hybridization: In Neurobiology; Advances in Methodology* (J. H. Eberwine, K. L. Valentino, and J. D. Barchas, eds., Oxford University Press, 1994); or *In Situ Hybridization: A Practical Approach* (D. G. Wilkinson, ed., Oxford University Press, 1992)).

The preferred embodiment of the probes and probe collections of the present invention are best understood by referring to Figures 1-21 and Examples 1-2. The Examples, which follow, are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

## EXAMPLE 1

### Probe Collection Preparation

Probe collections consisting of a plurality of oligonucleotide probes of 55 to 60 bases in length were designed as follows. In this Example, each oligonucleotide probe possessed two distinct domains: a 5' (or labeling) domain and a 3' (or target gene-specific) domain (*See* Figure 1).

In this embodiment, the labeling domain consists of the sequence (CTATTTT)n, wherein the cytosine nucleotide represents a fluorescein-cytosine nucleotide conjugate

and the fluorescein molecule is linked at the N4 position of cytosine through an OBEA linkage.

The target gene-specific domain consists of a 25-30 base sequence that is complementary to a specific nucleic acid target gene. Oligonucleotide probes were designed to possess target gene-specific domains corresponding to the human immunoglobulin kappa light chain variable region (*See* Table 1; oligonucleotide probes 401-416), the human immunoglobulin lambda light chain variable region (oligonucleotide probes 501-515), human cytomegalovirus (CMV) sequences (oligonucleotide probes 221-241), human Esptein-Barr virus (EBV) EBER (Epstein-Barr early RNA) sequences (oligonucleotide probes 100A2, 100C2, 100A1, and 100B1), human repetitive Alu sequences (oligonucleotide probes 301 and 302), and poly d(T) (oligonucleotide probe 320).

**EXAMPLE 2**

**Label-domain design: Alu repetitive sequence probe**

Four probes all against the Alu human repetative sequence were used to evaluate label-domain design. The probes numbered 301 (SEQ ID NO: 55), 301A (SEQ ID NO:116), 301A2/2 (SEQ ID NO: 121), and 301A3/2 (SEQ ID NO: 122) are shown in Table 1.

The four probes were evaluated at the concentrations of 100, 75, 50, and 25 ng/ml per mL of probe in the reaction, respectively. This hybridization analysis was done manually, using standard protocols. The target, paraffin-embedded cell line MBA MD 468 (Oncor INFORM™ Her-2/neu Control Slides, Cat. No. S8100, Level 1, available from Ventana Medical Systems, Inc., Tucson, AZ) was the target sample and was processed by removing paraffin by standard xylene methods. The tissue was subjected to Ventana's. Protease 1 for 12 minutes at 50 degrees C as a 1:2 dilution with Ventana's APK buffer. The hybridization reaction was accomplished with the addition of probe diluent as 100 ul probe (25% formamide, 5% dextran sulfate, 2X SSC, 1% Triton) to a residual 100 ul volume of 2XSSC/Triton X-100. The slide was heated to 85 degrees C for 5 minutes and then incubated for 1 hr at 37 degrees C. Standard SSC washes followed for removing excess probe. The hybrids were detected with an antibody against FITC. The mouse antibody was detected colormetrically using Ventana Enhanced Alkaline Phosphatase Blue Detection (cat# 760-061). Unless otherwise indicated, all reagents

were obtained from Ventana Medical Systems, Inc., Tucson, AZ. The results were observed by colormetric detection using brightfield microscopy.

The results of these experiments were that signal intensity was a function of the total number of fluorescein hapten conjugated to the probe and signal was of the specific label-domain design. The greater the number of fluoresceins per probe molecule, the greater the signal observed. Comparison of design and placement of haptens on the probe showed that this was not a factor in signal intensity. The two probes that contained five fluoresceins, (301A3/2 (SEQ ID NO: 122) and 301 (SEQ ID NO:55) both yielded equivalent signal. These two probes yielded greater signal that seen for 301A2/2, a probe with a split label-domain design with four fluoresceins. The probe 301A2/2 yielded a signal greater than probe 301A a probe with a single label-domain design at the 5' end and with three fluoresceins.

**EXAMPLE 3**

**Label Domain Design: EBER probes**

This experiment compared two label-domain designs and sequences to determine whether greater spacing between the fluorescein haptens improves the production of signal during probe detection steps during *in situ* hybridization analysis.

The tissue used was an EBV-infected human spleen tissue fixed in neutral buffered formalin paraffin embedded section of 4-micron thickness placed on silane plus glass microscope slides. The tissue sections were deparaffinized on a Ventana DISCOVERY™ machine, followed by a 6-min digestion with Ventana's Protease 1, at a temperature of 37 C. The probe was dissolved in hybridization buffer diluent at a concentration of 50 ng/mL as a 100 ul applied to an equal volume of 2 X SSC/ Triton X-100 residual volume left on the slide after prepared by the Ventana Medical Systems, Inc. automated ISH staining system, Discovery. The probe diluent-mixed with the residual volume on slide for 6 min at 37 C, then the solution was heated to 85C and held there for a total of 10 min. The slide was then taken to a 37C temperature and held at that temperature for 1 hour. All of these aqueous reactions on the slide were all done under a film of LIQUID COVERSLIP™, to prevent evaporative loss of water during processing. Each slide after hybridization was washed 3 times with 2X SSC/Triton solution, with a 6 min incubation between each wash, the slide volume being approximately 300 ul (+/- 10% vol). The hybrids were detected with an antibody against FITC. The mouse

antibody was detected colormetrically using Ventana Enhanced Alkaline Phosphatase Blue Detection (cat# 760-061).

The two oligonucleotide probes used for this study probe 100A1 (SEQ ID NO: 53) and 1002A32 (SEQ ID NO: 120). The two differences between these probes were the label-domain seqence and structure. The probe 100A1 label domain was 5' to gene target domain, contained 5 fluoresceins attached to cytosine residues via the OBEA linker, with the sequence formula of $(CTATTTT)_4CT$ (SEQ ID NO: 58). The label domain of the oligo probe 1002A32, was similiar, (SEQ ID NO:125). Besides the different sequence the primary difference was that the fluoresceine labeled cytosines were spaced 10 bases apart compared to the oligo 100A1 the cytosine spacing was closer at 7 bases apart. The result of this comparison as deduced by H score analysis were that these oligonucleotide were equivalent as to the amount of signal generated on the slide. The data was that for 100A2, for the 368 cells analysed in a total of 3 fields the H score was 106, and for probe 1002A32 for the 345 cell analysed in three field the H score was 109. The H score is a spectrographic analysis done with micrscope that factors into the score background to signal ratio on the tissue section to yield a relative comparison of total target specific signal on the slide. (See reference Giroud, F. Perrin C, and Simony Lafontaine, J.; Quantitative Immunocytochemistry and Immunohistochemistry. Third Conference of the European Society for Analytical Cellular Pathology, 1994; and AutoCyte Quic Immuno User's Manual, 1998, document number PA-029, Co AutoCyte Inc. Burlington NC 2721). The histograms and the score sheet indicated that each oligo were equally efficient in yielding a colormetric signal. This indicates that the position of the label domain can be either 3 prime or 5 prime to the gene target sequence or the gene target sequence can be positioned between two label domains.

**EXAMPLE 4**

***In Situ* Hybridization**

The probe collections prepared in Example 1 were first diluted in a solution consisting of 20% dextran sulfate (wt/vol), 50% formamide (vol/vol), 2X SSC, 10 mM Tris-HCl, 5 mM EDTA, and 0.05% Brij-35, at a final pH of 7.3. Probe collections were then mixed with an equal volume of a solution consisting of 2X SSC and 0.05% Triton X-100.

Samples for ISH analysis were prepared by cutting formalin-fixed and paraffin-embedded cells or tissue samples into 4 μm sections and placing the sections onto a glass slide. Subsequent processing and ISH of samples was carried out in an automated device, such as the DISCOVERY™ Automated ISH/IHC Stainer (Ventana Medical Systems, Inc., Tucson, AZ) described in co-owned and co-pending U.S. Patent App. Serial Nos. 60/076,198 and 09/259,240, both incorporated herein by reference. . To remove paraffin from the samples, the slides were immersed in an aqueous solution, heated for approximately 20 minutes, and then rinsed. The automated deparaffinization procedure is more fully described in U.S. Serial No. 60/099,018, 09/259,240 both incorporated herein by reference. The samples were then treated with protease and the slides were heated to 85°C (for hybridization to RNA target genes) or 90-95°C (for hybridization to DNA target genes) for 4 to 10 minutes.

Hybridization reactions were typically performed in a hybridization buffer consisting of 10% dextran sulfate (wt/vol), 25% formamide (vol/vol), 2X SSC, 5 mM Tris, 2.5 mM EDTA, 0.025% Brij-35, 0.25% Triton X-100, and between 25 to 125 ng/mL of each individual probe molecule. ISH reactions were performed at between 37°C to 54°C. For ISH using the probe collections described in Example 1, hybridization reactions were optimally carried out for 1 hr at 47°C (except for the poly d(T) probe, wherein the hybridization reaction was optimally carried out at 37°C for 1 hr).

The hybridization of fluorescein-labeled probe molecules to a particular target gene in the sample was detected by using a sequential series of binding proteins, i.e., secondary antibody detection. However, it is equally possible to use detect detection when visualizing the bound probes. In secondary detection, first, an anti-fluorescein mouse monoclonal antibody directed against the fluorescein-labeled probe molecule was added to the sample. Next, a biotin-labeled polyclonal goat antibody directed against the mouse antibody was added to the sample. Finally, hybridization reactions were colormetrically detected using a 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) substrate This technique, termed "secondary antibody detection," is routine for one of skill in the art. Primary and secondary antibodies are available from numerous suppliers, including Ventana Medical Systems, Tucson, AZ, which are optimized for use on the Ventana autostaining systems (ES®, NexES®, DISCOVERY™, and BENCHMARK™.

Figures 2-21 illustrate the results obtained for *in situ* hybridization analysis of various cell lines or tissue samples using the probes disclosed and claimed herein having the structural motif illustrated in Figure 1 or probe collections consisting of such probes.

Figure 1 illustrates a generic probe structure of of the two-domain probe design. This is the oligonucleotide design used for the probes in the gene specific cocktails described in the following examples. Each probe is composed of two domains: a 5' labeling domain and a 3' target gene target gene-specific domain. The labeling domain consists of this specific sequence (CTATTTT)n, wherein the cytosine nucleotide is a cytosine-hapten conjugate, the hapten being fluorescein in this embodiment. This illustration specifically shows nucleic acid sequences for the 301 (SEQ ID NO: 55) and 302 (SEQ ID NO: 56) probes, each of which possesses target gene gene-specific domains corresponding to human repetitive Alu sequences and labeling domains having a fluorescein hapten.

Figure 2 illustrates the results obtained for *in situ* hybridization (ISH) analysis of human skin tissue using a probe comprising the labeling domain (330 probe; SEQ ID NO: 58). The absence of a detectable signal indicates that the sequence formula, (CTATTTT)n, of the labeling domain common to the oligonucleotides used in these ISH examples is non-specific, and non-reactive in its ability to form Watson-Crick base pairing with human nucleic acid sequences because it does not hybridize.

Figure 3 illustrates the results obtained for ISH analysis of human skin tissue using a probe comprising the labeling domain and a poly d(T) target gene-specific domain (320 probe; SEQ ID NO: 57). The presence of a detectable signal localized to the cytoplasm indicates that this probe is capable of specifically hybridizing to polyadenylated region of messenger RNA.

Figures 4A-4B illustrate the results obtained for ISH analysis of human skin tissue using the 320 probe, wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The decrease in detectable signal in (B) indicates that this probe specifically hybridizes to polyadenylated region common to messenger RNA. .

Figures 5A-5B illustrate the results obtained for ISH analysis of human spleen tissue using the 320 probe, wherein the hybridization and stringency wash were performed at room temperature (A), or at 37°C (B). This result illustrates that the

intenstity of color is related to the stringency of hybridization conditions, with the more intense color indicating less stringent conditons.

Figure 6 illustrates the results obtained for ISH analysis of the human Raji cell line using the 320 probe. This shows that this probe design also is functional with embedded cell lines as well as embedded tissue.

Figure 7 illustrates the results obtained for ISH analysis of the human Raji cell line using a probe collection consisting of the 301 and 302 probes.

Figure 8 illustrates the results obtained for ISH analysis of the human HT cell line using a probe collection consisting of the 301 and 302 probes.

Figure 9 illustrates the results obtained for ISH analysis of a rat cell line using a probe collection consisting of the 301 and 302 probes. The absence of a detectable signal indicates that this probe collection is specific for human nucleic acid sequences.

Figure 10 illustrates the results obtained for ISH analysis of an Epstein-Barr virus (EBV)-negative human HT cell line using a probe possessing a target gene-specific domain corresponding to EBV EBER nuclear RNA [SEQ ID NO: 51 through SEQ ID NO: 54].

Figure 11 illustrates the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figure 12 illustrates the results obtained for ISH analysis of human tonsil tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figures 13A-13B illustrate the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54, wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The decrease in detectable signal in (B) indicates that this probe specifically hybridizes to human EBER 1 and EBER 2 nuclear RNA .

Figure 14 illustrates the results obtained for ISH analysis of kappa light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NO:15].

Figure 15 illustrates the results obtained for ISH analysis of lymphoma tissues using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin kappa light chain mRNA [SEQ ID NOS: 2 - 4, SEQ ID NOS:7-12, SEQ ID NOS: 14, 15]. The lymphoma tissue in (A) over expresses the kappa light chain and the tissue in (B) over expresses the lambda light chain. The absence of a detectable signal in (B) indicates that the kappa light chain probe collection is specific to kappa light chain mRNA.

Figure 16 illustrates the results obtained for ISH analysis of lambda light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain variable region mRNA [SEQ ID NOS:19 through 29].

Figure 17 illustrates the results obtained for ISH analysis of a lambda light chain-positive human RPMI 8226 cell line using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NOS:19 through 29].

Figures 18A-18B illustrate the results obtained for ISH analysis of human lymphoma tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NOS:19 through 29]. The tissue in (A) over expresses the lambda light chain and the tissue in (B) over expresses the kappa light chain. The absence of a detectable signal in (B) indicates that the lambda light chain probe collection is specific to human lambda light chain mRNA.

Figure 19 illustrates the results obtained for ISH analysis of cytomeglovirus (CMV)-positive human lung tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50]. Arrow indicates CMV infected cell.

Figure 20 illustrates the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has not been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-

specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50].

Figures 21A-21B illustrate the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50] expression of the CMV immediate early RNA with cyclohexamide. The tissue in (A) is shown at a magnification of 40X and the tissue in (B) is shown at a magnification of 20X.

**TABLE 1**

| Probe ID | Sequence | SEQ ID |
|---|---|---|
| 401 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCAGAGTAGCAGGAGCCCCAGGAGCTGAGC-3' | 1 |
| 402 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGATGGAGACTGGGTCAACTGGATGTCACA-3' | 2 |
| 403 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GCAAGCGATGGTGACTCTGTCTCCTACAGC-3' | 3 |
| 404 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TCTGTCCCAGATCCACTGCCACTGAACCTT-3' | 4 |
| 405 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GCAGCCACAGTTCGCTTCATCTGCACCTTG-3' | 5 |
| 406 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TTTCAACTGCTCATCAGATGGCGGGAAGAT-3' | 6 |
| 407 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AAGTTATTCAGCAGGCACACAACAGAGGCA-3' | 7 |
| 408 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGCGTTATCCACCTTCCACTGTACTTTGGC-3' | 8 |
| 409 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TAGGTGCTGTCCTTGCTGTCCTGCTCTGTG-3' | 9 |
| 410 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GTAGTCTGCTTTGCTCAGCGTCAGGGTGCT-3' | 10 |
| 411 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GATGGGTGACTTCGCAGGCGTAGACTTTGT-3' | 11 |
| 412 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CTCTCCCCTGTTGAAGCTCTTTGTGACGGG-3' | 12 |
| 413 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TGGAACTGAGGAGCAGGTGGGGGCACTTCT-3' | 13 |
| 414 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GAAAAAGGGTCAGAGGCCAAAGGATGGGAG-3' | 14 |
| 415 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AGATGAGCTGGAGGACCGCAATAGGGGTAG-3' | 15 |
| 416 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GCATAATTAAAGCCAAGGAGGAGGAGGGGG-3' | 16 |
| 501 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCTGAGTGAGGAGGGTGAGGAGCAGCAGAG-3' | 17 |
| 502 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AGACCCAGACACGGAGGCAGGCTGAGTCAG-3' | 18 |
| 503 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TGTTGGTTCCAGTGCAGGAGATGGTGATCG-3' | 19 |
| 504 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TAAATCATGATTTTGGGGGCTTTGCCTGGG-3' | 20 |

| | | | |
|---|---|---|---|
| 505 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TGTTGCCAGACTTGGAGCCAGAGAAGCGAT-3' | 21 |
| 506 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AATAATCAGCCTCGTCCTCAGCCTGGAGCC-3' | 22 |
| 507 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GGTCCCTCCGCCGAAAACCACAGTGTAACT-3' | 23 |
| 508 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TTATGAGACACACCAGTGTGGCCTTGTTGG-3' | 24 |
| 509 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CTGCTCAGGCGTCAGGCTCAGATAGCTGCT-3' | 25 |
| 511 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ATGCGTGACCTGGCAGCTGTAGCTTCTGTG-3' | 26 |
| 512 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ATTGTGTAGGGGCCACTGTCTTCTCCACGG-3' | 27 |
| 513 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CCTCCCCTGGGATCCTGCAGCTCTAGTCTC-3' | 28 |
| 515 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TGAGGGTTTATTGAGTGCAGGGAGAAGGGC-3' | 29 |
| 221 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GGAGGTCAAAACAGCGTGGATGGCG-3' | 30 |
| 222 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GAGGCTGGATCGGTCCCGGTGTCTT-3' | 31 |
| 223 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AATCCGCGTTCCAATGCACCGTTCC-3' | 32 |
| 224 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TAAAAACTGCGGGCACTGGGGACGG-3' | 33 |
| 225 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ACCCGAGATTCGCGTGGAGATCCCA-3' | 34 |
| 226 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GAGCAAGGAGCTGCCGAGCGACCAT-3' | 35 |
| 227 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ACACTGGTGGTGGTGGGCATCGTGC-3' | 36 |
| 228 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TTCCAAATGCGTCAGCGGTGCAAGC-3' | 37 |
| 229 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AGCTGCCTGCATCTTCTTCTGCCGC-3' | 38 |
| 230 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CCCTCCACCGTTAACAGCACCGCAA-3' | 39 |
| 231 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TTGGTCACGGGTGTCTCGGGCCTAA-3' | 40 |
| 232 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TCGGCCAACTCTGGAAACAGCGGGT-3' | 41 |
| 233 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TCGGGGTTCTCGTTGCAATCCTCGG-3' | 42 |
| 234 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ATCTCGATGCCCCGCTCACATGCAA-3' | 43 |
| 235 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TGCCGCACCATGTCCACTCGAACCT-3' | 44 |
| 236 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GTTAGCGGCGCCCTTGCTCACATCA-3' | 45 |
| 237 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TGCAGATCTCCTCAATGCGGCGCTT-3' | 46 |
| 238 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TCTCAGAGGATCGGCCCCCAGAATG-3' | 47 |
| 239 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CCTCATCTGACTCCTCGGCGATGGC-3' | 48 |
| 240 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CGGGTACAGGGGACTCTGGGGGTGA-3' | 49 |
| 241 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GGGTGGGTGCTCTTGCCTCCAGAGG-3' | 50 |
| 100A2 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GACCTCGGGTCGGTAGCACCGCACT-3' | 51 |
| 100C2 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GGAAGCCTCTCTTCTCCTCCCCCGG-3' | 52 |
| 100A1 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CCACAGACACCGTCCTCACCACCCG-3' | 53 |

| | | |
|---|---|---|
| 100B1 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGCTACAGCCACACACGTCTCCTCC-3' | 54 |
| 301 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGAGGCGGGCGGATCACCTGAGGTC-3' | 55 |
| 302 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 56 |
| 320 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TTTTTTTTTTTTTTTTTTTTTTTTTT-3' | 57 |
| 301A | 5'-CTATTTTCTATTTTCTTTT CGAGGCGGGCGGATCACCTGAGGTC-3' | 116 |
| 302C | 5'-CTATTTTCTATTTTCTTTT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 117 |
| 302A4 | 5'-CTATTTTATACTTTATATTTCATATTTTATCT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 118 |
| 302A3/2 | 5'-CTATTTTATATTTATATTTCT CGGGAGGCGGAGGTTGCAGTGAGCC ACTATTTTATACTT-3 | 119 |
| 1002A32 | 5'-CTATTTTATACTTTATATTTCT GACCTCGGGTCGGTAGCACCGCAC TACTATTTTATACTT-3' | 120 |
| 301A2/2 | 5'-CTATTTTTCTT CGAGGCGGGCGGATCACCTGAGGTC TTCTTTTTATCTT-3 | 121 |
| 301A3/2 | 5'-CTATTTTATACTTTATATTTCT CGAGGCGGGCGGATCACCTGAGGTC ACTATTTTATACTT-3' | 122 |

**Table 2**

| Probe ID | Sequence | SEQ ID |
|---|---|---|
| | 5'-CTATTTTTCTATTTTTCTTTT | 123 |
| | 5'-CTATTTTATACTTTATATTTCATATTTTATCT | 124 |
| 330 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | 58 |
| | 5'-CTATTTTATACTTTATATTTCT.....ACTATTTTATACTT-3 | 125 |
| | 5'-CTATTTTTCTT.....TTCTTTTTATCTT-3 | 126 |

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

WE CLAIM:

1. An oligonucleotide label-domain comprising the sequence $(CTATTTT)_n$ and its complement $(AAAATAG)_n$ wherein "n" is at least 1.

2. The oligonucleotide label-domain of claim 1 detectably labeled with a reporter molecule, or a hapten molecule.

3. The oligonucleotide label-domain of claim 2 wherein the hapten is flurorescein linked to the N4 nitrogen of cytosine through an OBEA linker.

4. The oligonucleotide label-domain of claim 1 wherein the reporter molecule is a fluorophore.

5. The oligonucleotide label-domain of claim 1 wherein the fluorophore is present at a density of greater than 7 mole percent.

6. The oligonucleotide label-domain of claim 1 wherein the label-domain has the sequence $TC(TTTTATC)_n$ ( or its complementary formula).

7. The oligonucleotide label-domain of claim 1 wherein the sequence is SEQ ID NO: 58.

8. The oligonucleotide label-domain of claim 2 wherein at least 7 mole pecent of the cytosines are linked to a detectable moiety by an OBEA linker.

9. An oligonucleotide probe having at least two distinct functional domains, a first domain comprising the label-domain of claim 2, and a second domain comprising a gene-specific target sequence.

10. The oligonucleotide probe of claim 9 wherein the label-domain is located at the 5' end of the oligonucleotide probe, and the gene-specific target sequence being 3' to the label-domain.

11. The oligonucleotide probe of claim 9 wherein the label-domain is located at the 3' end of the oligonucleotide probe, and the gene-specific target sequence is 5' to the label-domain.

12. An oligonucleotide probe having three distinct functional domains, a first domain comprising the label-domain of claim 2, a second domain comprising a gene-specific target sequence, and a third domain comprising another label-domain, wherein said second domain is located between said first and third domains.

13. A probeset for detecting Kappa immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 401 through 416, inclusive.

14. A probeset for detecting Lambda immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 501 through 509, 511-513, and 515.

15. A probeset for detecting cytomegalovirus (CMV) immediate early RNA and /or corresponding mRNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 221 through 241.

16. A probeset for detecting Epstein Barr virus (EBV) early RNA, RNA 1 and RNA 2, (EBER) wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 51 through 54.

17. A probeset for detecting Human Alu repetitive sattelite genomic DNA sequences wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 301 and 302.

Oligonucleotide Probe Design
Common Signal Detection Region
Unique Target Detection Region
5'-
-3'
Alu301 5'-CTATTTTCTATTTTCTATTTTCT-CGAGGCGGGCGGATCACCTGAGGTC-3'
Alu302 5'-CTATTTTCTATTTTCTATTTTCT-CGGGAGGCGGAGGTTGCAGTGAGCC-3'

FIG. 1

FIG. 2

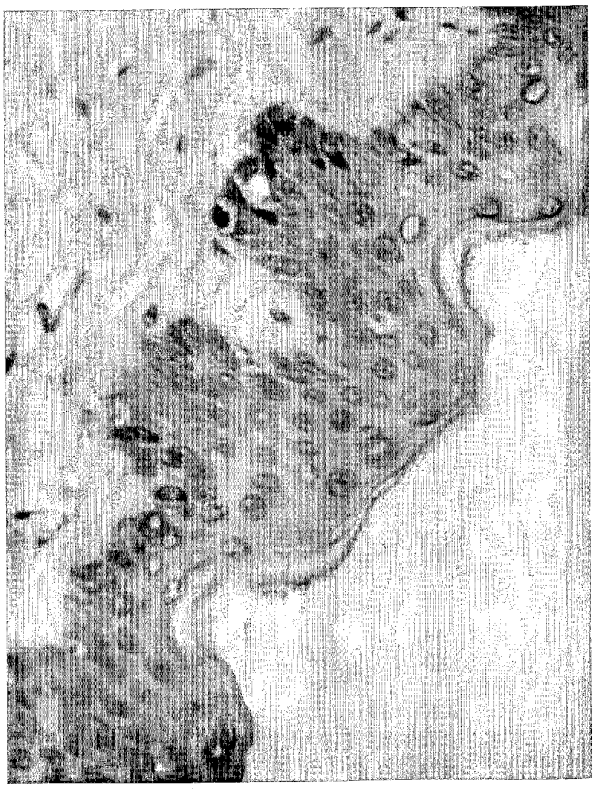

FIG. 3

PLUS RNase

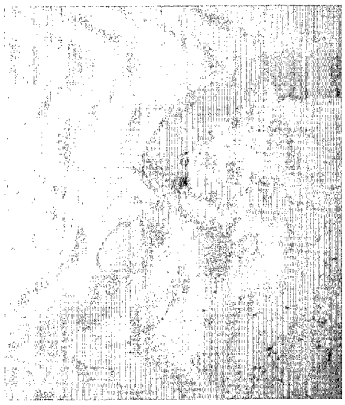

FIG. 4B

MINUS RNase

FIG. 4A

37/37

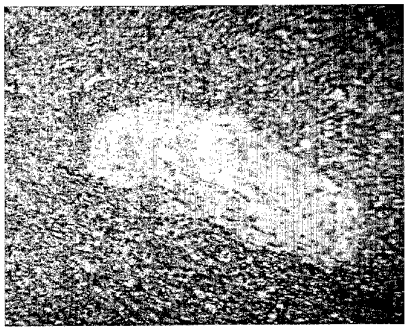

FIG. 5B

RT/RT

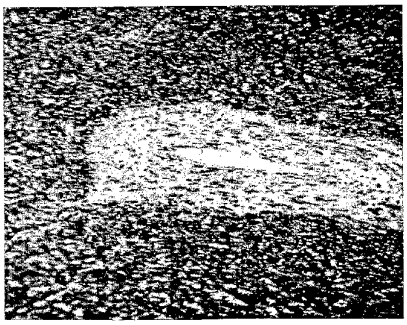

FIG. 5A

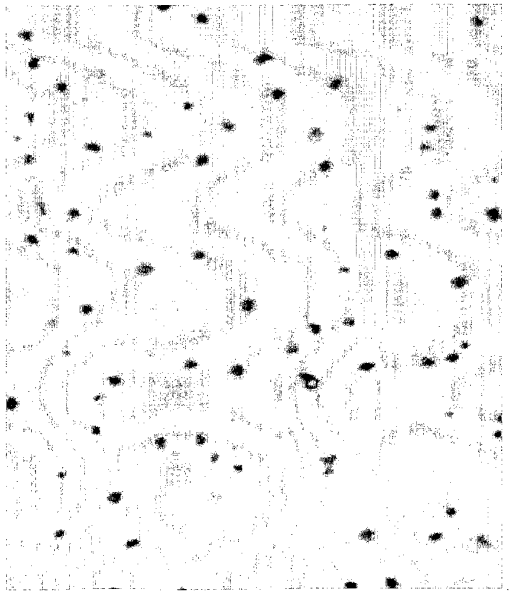

FIG. 6

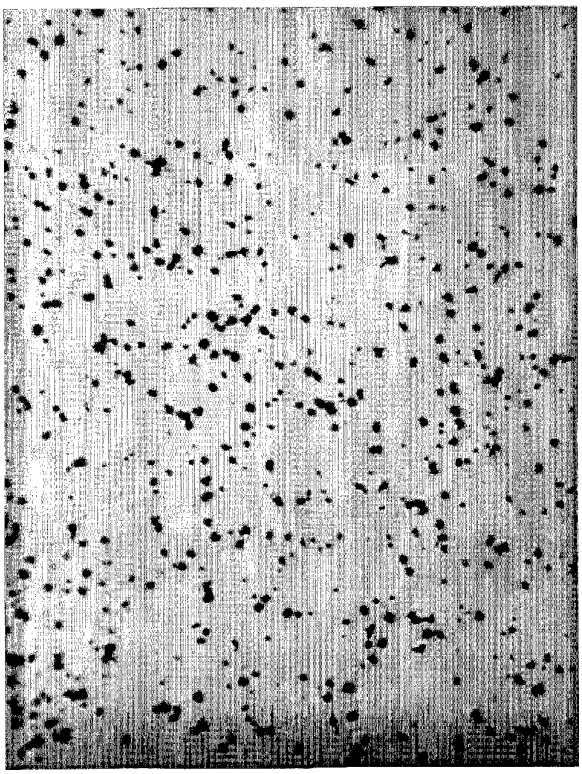

FIG. 7

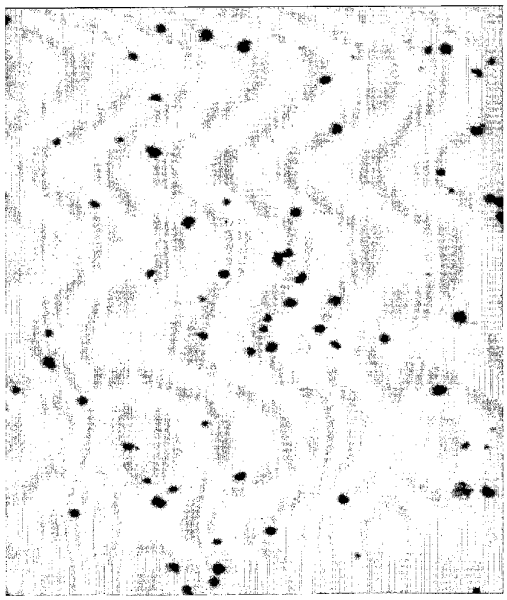

FIG. 8

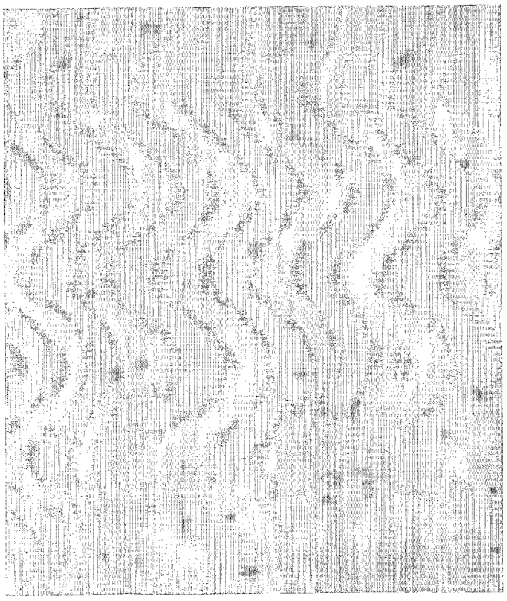

FIG. 9

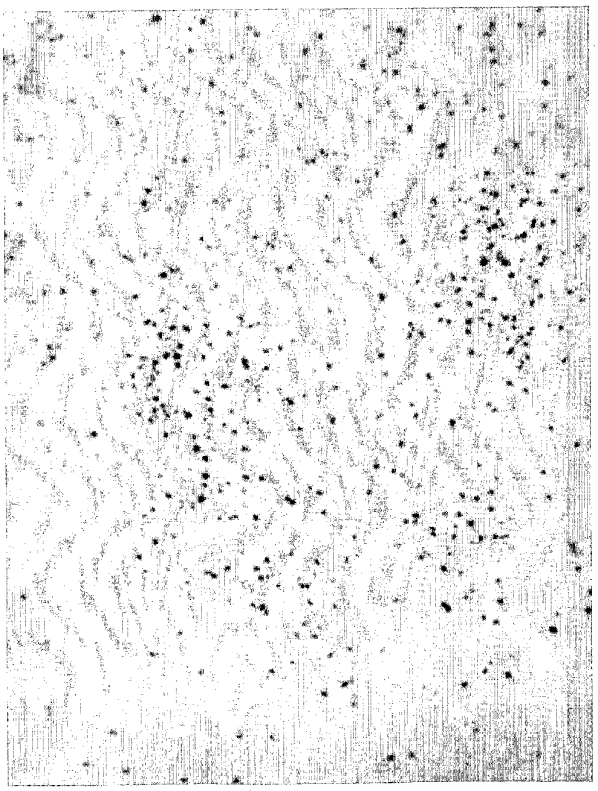

FIG. 11

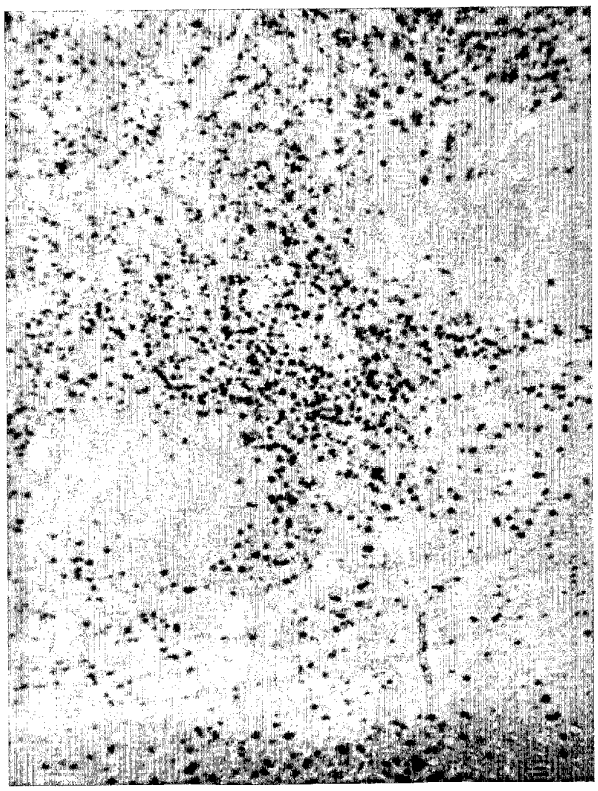

FIG. 12

PLUS RNase

FIG. 13B

MINUS RNase

FIG. 13A

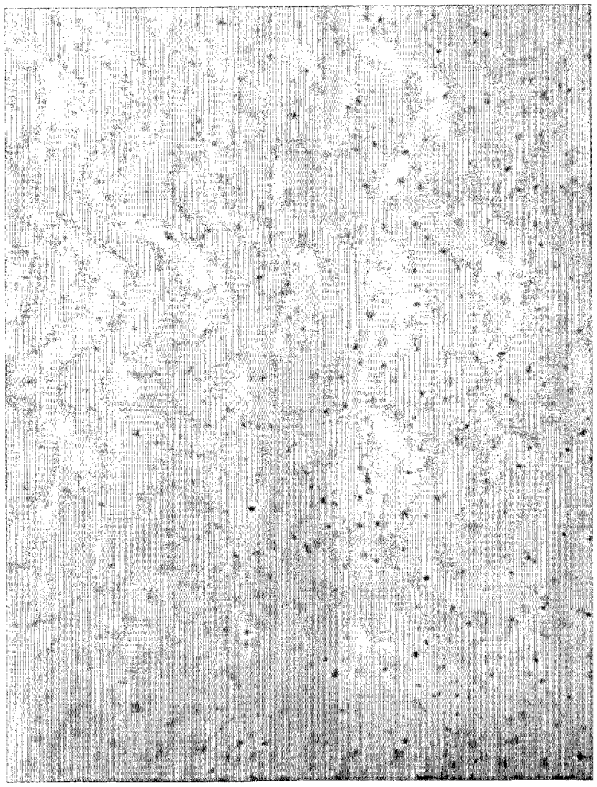

FIG. 10

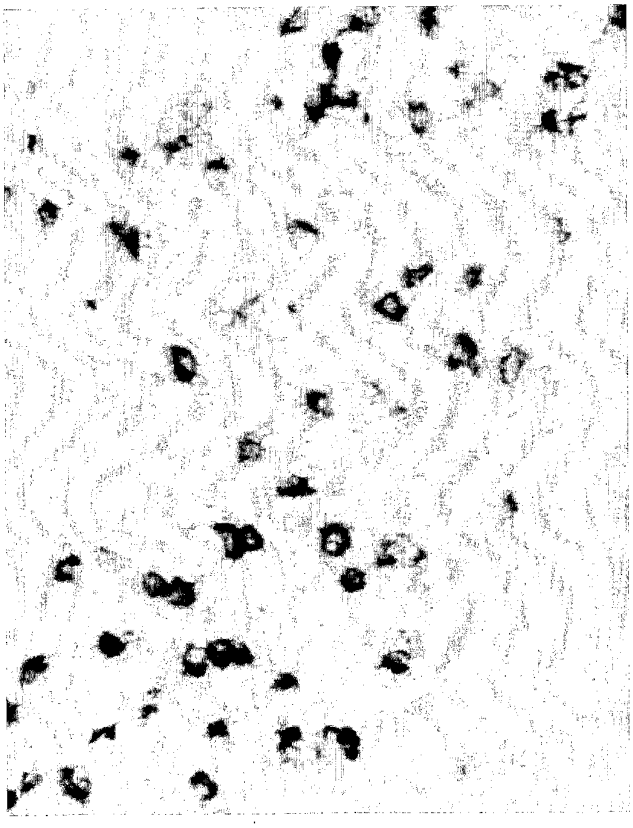

FIG. 14

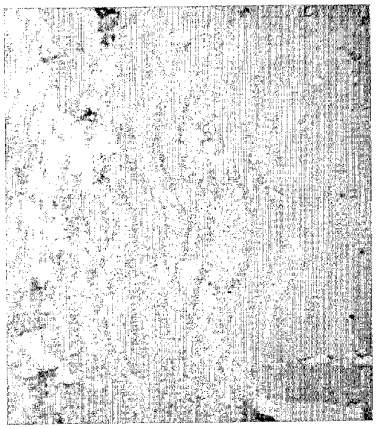

FIG. 15B

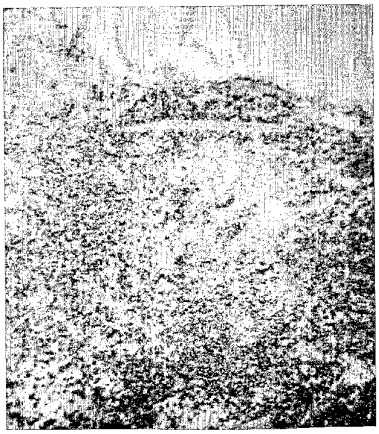

FIG. 15A

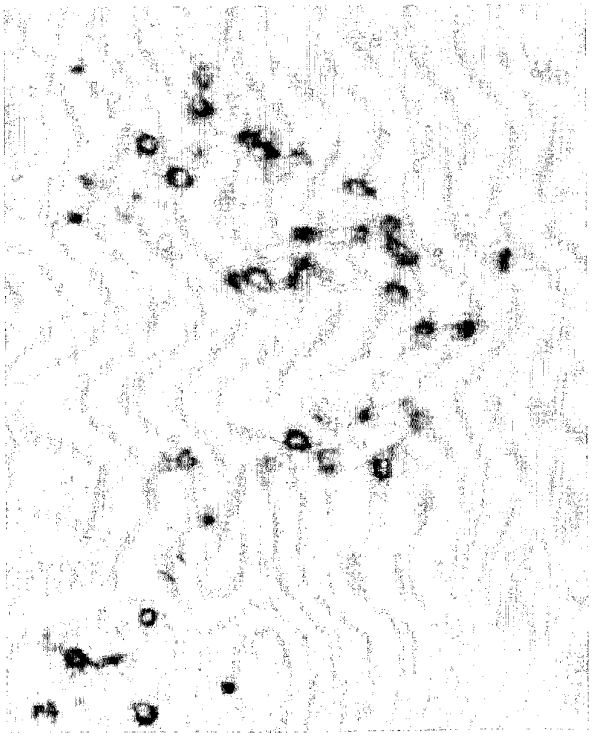

FIG. 16

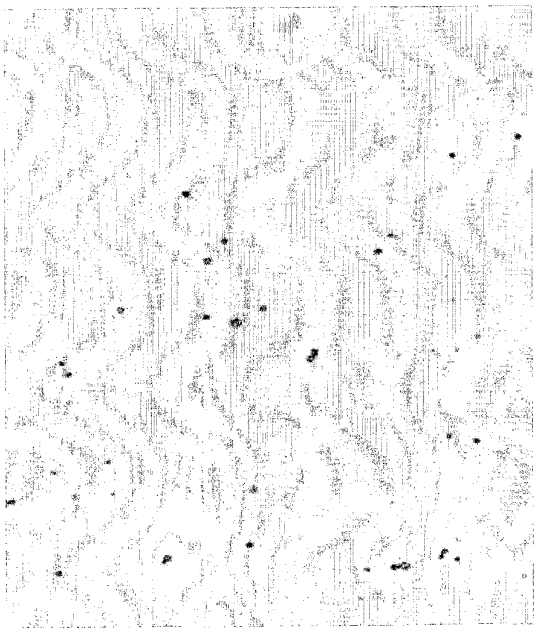

FIG. 17

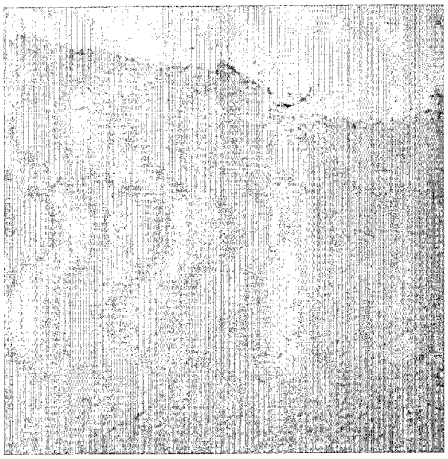

FIG. 18B

FIG. 18A

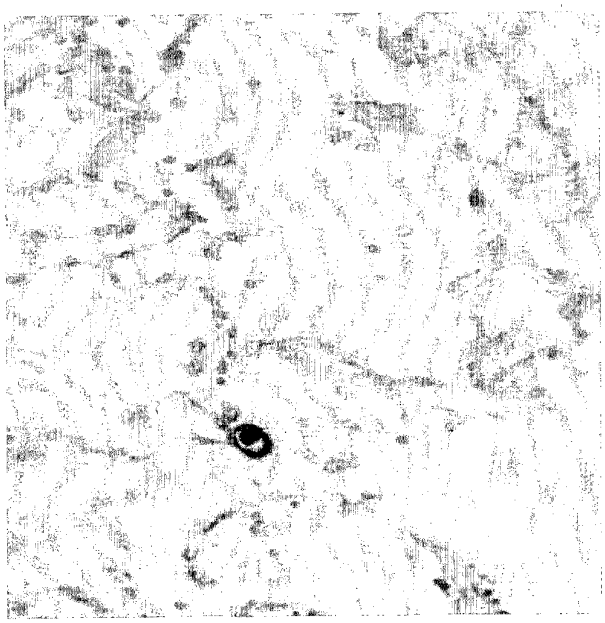

FIG. 19

FIG. 20

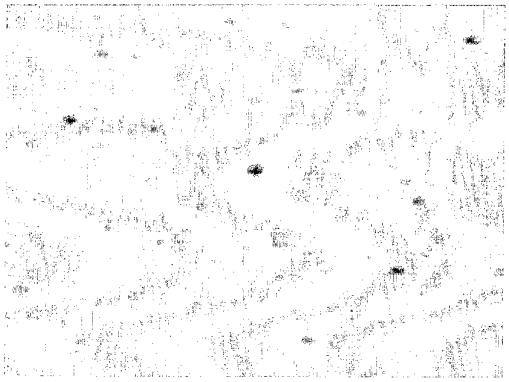

FIG. 21B

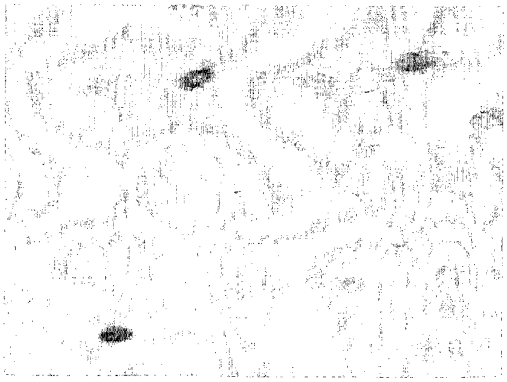

FIG. 21A

SEQUENCE LISTING

<110> Utermohlen, Joseph
<110> Connaughton, John

<120> Oligonucleotide sequence formula for labeling Oligonucleotide Probes and Proteins for *In Situ* Analysis

<130> 355/001/PCT

<140>
<141> 2001-09-06
<150> 60/233,177
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<160> 126

<170> PatentIn Ver. 2.0

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Oligonucleotide probe

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<211> 60
<212> DNA
<213> Artificial Sequence

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Oligonucleotide probe

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<210> 3
<211> 60
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<220>
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Oligonucleotide probe

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<210> 4

<211> 60
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Oligonucleotide probe

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ctatttcta ttttctattt tctatttttct tctgtcccag atccactgcc actgaacctt 60

<210> 5
<211> 60
<212> DNA
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<220>
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Oligonucleotide probe

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ctatttcta ttttctattt tctatttttct gcagccacag ttcgcttcat ctgcaccttg 60

<210> 6
<211> 60
<212> DNA
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<223> Description of Artificial Sequence:
Oligonucleotide probe

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<210> 7
<211> 60
<212> DNA
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<223> Description of Artificial Sequence:
Oligonucleotide probe

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ctatttcta ttttctattt tctatttttct aagttattca gcaggcacac aacagaggca 60

<210> 8
<211> 60
<212> DNA
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<223> Description of Artificial Sequence:
Oligonucleotide probe

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ctatttcta ttttctattt tctatttttct ggcgttatcc accttccact gtactttggc 60

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<211> 60
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<223> Description of Artificial Sequence:
Oligonucleotide probe

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ctatttcta ttttctattt tctatttttct taggtgctgt ccttgctgtc ctgctctgtg 60

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<211> 60
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Oligonucleotide probe

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<210> 11
<211> 60
<212> DNA
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

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<211> 60
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<220>
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Oligonucleotide probe

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<211> 60
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<220>
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Oligonucleotide probe

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<210> 14
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
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Oligonucleotide probe

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<210> 15
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

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<210> 16
<211> 60
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<220>
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Oligonucleotide probe

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ctatttcta ttttctattt tctattttct gcataattaa agccaaggag gaggaggggg 60

<210> 17
<211> 60
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Oligonucleotide probe

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<210> 18
<211> 60
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

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<210> 19
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
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Oligonucleotide probe

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<210> 20
<211> 60
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Oligonucleotide probe

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<210> 21
<211> 60
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Oligonucleotide probe

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<210> 22
<211> 60
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Oligonucleotide probe

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<210> 23

<211> 60
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<220>
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<210> 26
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<212> DNA
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Oligonucleotide probe

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<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 27

ctatttcta ttttctattt tctattttct attctgtagg ggccactgtc ttctccacgg 60

<210> 28
<211> 60
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<220>
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Oligonucleotide probe

<400> 28
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<210> 29
<211> 60
<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 29
ctatttcta ttttctattt tctattttct tgagggttta ttgagtgcag ggagaagggc 60

<210> 30
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 30
ctatttcta ttttctattt tctattttct ggaggtcaaa acagcgtgga tggcg 55

<210> 31
<211> 55
<212> DNA
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 31
ctatttcta ttttctattt tctattttct gaggctggat cggtcccggt gtctt 55

<210> 32
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:

Oligonucleotide probe

<400> 32
ctattttcta ttttctattt tctatttttct aatccgcgtt ccaatgcacc gttcc 55

<210> 33
<211> 55
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<223> Description of Artificial Sequence:
Oligonucleotide probe

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ctattttcta ttttctattt tctatttttct taaaaactgc gggcactggg gacgg 55

<210> 34
<211> 55
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Oligonucleotide probe

<400> 34
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<210> 35
<211> 55
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<220>
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Oligonucleotide probe

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ctattttcta ttttctattt tctatttttct gagcaaggag ctgccgagcg accat 55

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<400> 37
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<210> 38
<211> 55
<212> DNA
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<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 38
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<210> 39
<211> 55
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

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ctatttcta ttttctattt tctattttct ccctccaccg ttaacagcac cgcaa 55

<210> 40
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Oligonucleotide probe

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<210> 41
<211> 55
<212> DNA
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Oligonucleotide probe

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<210> 42

<211> 55
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Oligonucleotide probe

<400> 42
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<210> 43
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<223> Description of Artificial Sequence:
Oligonucleotide probe

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ctatttcta ttttctattt tctatttttct atctcgatgc cccgctcaca tgcaa 55

<210> 44
<211> 55
<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 44
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<210> 45
<211> 55
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 45
ctatttcta ttttctattt tctatttttct gttagcggcg cccttgctca catca 55

<210> 46
<211> 55
<212> DNA
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 46

ctatttteta ttttctattt tctatttttct tgcagatctc ctcaatgcgg cgctt 55

<210> 47
<211> 55
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 47
ctatttteta ttttctattt tctatttttct tctcagagga tcggccccca gaatg 55

<210> 48
<211> 55
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<223> Description of Artificial Sequence:
Oligonucleotide probe

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<210> 49
<211> 55
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<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 49
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Oligonucleotide probe

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<210> 51
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<223> Description of Artificial Sequence:

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<223> Description of Artificial Sequence:
Oligonucleotide probe

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<210> 53
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

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<210> 54
<211> 56
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Oligonucleotide probe

<400> 54
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<210> 55
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<223> Description of Artificial Sequence:
Oligonucleotide probe

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ctatttcta ttttctattt tctatttttct cgaggcgggc ggatcacctg aggtc 55

<210> 56
<211> 55
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

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<210> 57
<211> 60
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

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<210> 58
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<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 58
ctattttcta ttttctattt tctatttttct 30

<210> 59
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Oligonucleotide probe

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ccagagtagc aggagcccca ggagctgagc 30

<210> 60
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<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 60
ggatggagac tgggtcaact ggatgtcaca 30

<210> 61

<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 61
gcaagcgatg gtgactctgt ctcctacagc 30

<210> 62
<211> 30
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Oligonucleotide probe

<400> 62
tctgtcccag atccactgcc actgaacctt 30

<210> 63
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 63
gcagccacag ttcgcttcat ctgcaccttg 30

<210> 64
<211> 30
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 64
tttcaactgc tcatcagatg gcgggaagat 30

<210> 65
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<212> DNA
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 65

aagttattca gcaggcacac aacagaggca 30

<210> 66
<211> 30
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 66
ggcgttatcc accttccact gtactttggc 30

<210> 67
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Oligonucleotide probe

<400> 67
taggtgctgt ccttgctgtc ctgctctgtg 30

<210> 68
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 68
gtagtctgct ttgctcagcg tcagggtgct 30

<210> 69
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 69
gatgggtgac ttcgcaggcg tagactttgt 30

<210> 70
<211> 30
<212> DNA
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<220>
<223> Description of Artificial Sequence:

Oligonucleotide probe

<400> 70
ctctccctg ttgaagctct ttgtgacggg 30

<210> 71
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 71
tggaactgag gagcaggtgg gggcacttct 30

<210> 72
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 72
gaaaaagggt cagaggccaa aggatgggag 30

<210> 73
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<212> DNA
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 73
agatgagctg gaggacgcga ataggggtag 30

<210> 74
<211> 30
<212> DNA
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 74
gcataattaa agccaaggag gaggaggggg 30

<210> 75
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Oligonucleotide probe

<400> 75
cctgagtgag gagggtgagg agcagcagag 30

<210> 76
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<220>
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Oligonucleotide probe

<400> 76
agacccagac acggaggcag gctgagtcag 30

<210> 77
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<220>
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Oligonucleotide probe

<400> 77
tgttggttcc agtgcaggag atggtgatcg 30

<210> 78
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Oligonucleotide probe

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taaatcatga ttttgggggc tttgcctggg 30

<210> 79
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<220>
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Oligonucleotide probe

<400> 79
tgttgccaga cttggagcca gagaagcgat 30

<210> 80

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Oligonucleotide probe

<400> 80
aataatcagc ctcgtcctca gcctggagcc 30

<210> 81
<211> 30
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<220>
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Oligonucleotide probe

<400> 81
ggtccctccg ccgaaaacca cagtgtaact 30

<210> 82
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<220>
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Oligonucleotide probe

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ttatgagaca caccagtgtg gccttgttgg 30

<210> 83
<211> 30
<212> DNA
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 83
ctgctcaggc gtcaggctca gatagctgct 30

<210> 84
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61

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CTATTTTTCTTNNNNNNNNNNNNNNNNNNNNNNNNNNNTTCTTTTTATCTT 49

【国際公開パンフレット（コレクトバージョン）】

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 March 2002 (21.03.2002)

PCT

(10) International Publication Number
WO 02/022874 A2

(51) International Patent Classification⁷: C12Q 1/68

(21) International Application Number: PCT/US01/28014

(22) International Filing Date:
6 September 2001 (06.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/233,177 15 September 2000 (15.09.2000) US

(71) Applicant *(for all designated States except US)*: **VENTANA MEDICAL SYSTEMS, INC.** [US/US]; 3865 N. Business Center Drive, Tucson, AZ 85705 (US).

(72) Inventors; and
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(74) Agent: **JONES, Huw, R.**; Ventana Medical Systems, Inc., 1910 Innovation Park Drive, Tucson, AZ 85737 (US).

(81) Designated States *(national)*: AU, CA, JP, MX, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

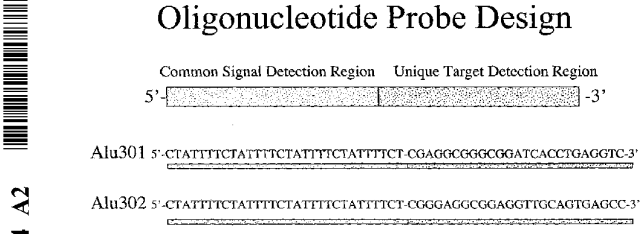
Published:
*without international search report and to be republished upon receipt of that report*

(48) Date of publication of this corrected version:
10 July 2003

(15) Information about Corrections:
see PCT Gazette No. 28/2003 of 10 July 2003, Section II

*[Continued on next page]*

(54) Title: OLIGONUCLEOTIDE SEQUENCE FORMULA FOR LABELING OLIGONUCLEOTIDE PROBES AND PROTEINS FOR *IN SITU* ANALYSIS




(57) Abstract: The present invention provides oligonucleotide probes and oligonucleotide probe collections and protein labeling for detecting or localizing a plurality nucleic acid target genes or antigens within a cell or tissue sample. Specifically, the provides collections of oligonucleotide probe for use in *in situ* hybridization analyses in which each probe has a label-domain with the sequence formulas of $(CTATTTT)_n$n?, $(AAAATAG)_n$n? or $(TTTTATC)_n$n? or $(GATAAAA)_n$n? in which all cases "n" would equal 1 or greater. The present invention provides collections or "cocktails" of oligonucleotide probes for detecting or localizing specific nucleic acid target genes within a cell or tissue sample. The cocktails are useful for detecting the following: the Kappa gene (SEQ ID NOS: 1-16 inclosive); the Lamba gene (SEQ ID NOS: 501-509, 511-513, and 515); the CMV (cytomegalovirus) gene (SEQ ID NOS: 221-241 inclusive); EBER (Epstein-Barr RNA) gene (SEQ IN NOS: 51-54 inclusive): Alu (SEQ IN NOS: 55-56); PolyA (SEQ ID NO: 57); and the detection tail (SEQ ID NO: 330).

WO 02/022874 A2

WO 02/022874 A2

**Previous Correction:**
see PCT Gazette No. 14/2003 of 3 April 2003, Section II

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

【国際公開パンフレット（コレクトバージョン）】

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 March 2002 (21.03.2002)

PCT

(10) International Publication Number
WO 02/022874 A3

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number: PCT/US01/28014

(22) International Filing Date:
6 September 2001 (06.09.2001)

(25) Filing Language: English

(26) Publication Language: English

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60/233,177 15 September 2000 (15.09.2000) US

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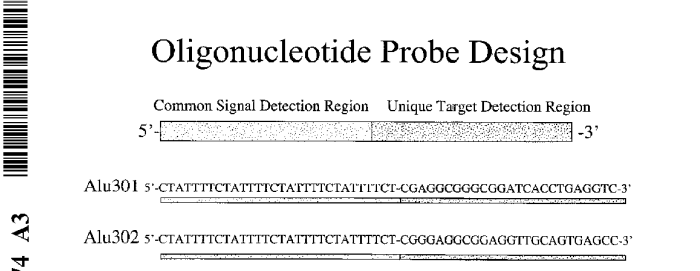
**Published:**
*with international search report*

(88) Date of publication of the international search report:
25 September 2003

(15) Information about Corrections:
**Previous Corrections:**
see PCT Gazette No. 28/2003 of 10 July 2003, Section II
see PCT Gazette No. 14/2003 of 3 April 2003, Section II

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: OLIGONUCLEOTIDES FOR LABELING OLIGONUCLEOTIDE PROBES AND PROTEINS




(57) Abstract: The present invention provides oligonucleotide probes and oligonucleotide probe collections and protein labeling for detecting or localizing a plurality nucleic acid target genes or antigens within a cell or tissue sample. Specifically, the provides collections of oligonucleotide probe for use in *in situ* hybridization analyses in which each probe has a label-domain with the sequence formulas of $(CTATTTT)_n$, $(AAAATAG)_n$ or $(TTTTATC)_n$ or $(GATAAAA)_n$ in which all cases "n" would equal 1 or greater. The present invention provides collections or "cocktails" of oligonucleotide probes for detecting or localizing specific nucleic acid target genes within a cell or tissue sample. The cocktails are useful for detecting the following: the Kappa gene (SEQ ID NOS: 1-16 inclusive); the Lamba gene (SEQ ID NOS: 501-509, 511-513, and 515); the CMV (cytomegalovirus) gene (SEQ ID NOS: 221-241 inclusive); EBER (Epstein-Barr RNA) gene (SEQ IN NOS: 51-54 inclusive): Alu (SEQ IN NOS: 55-56); PolyA (SEQ ID NO: 57); and the detection tail (SEQ ID NO: 330).

WO 02/022874 A3

【国際公開パンフレット（コレクトバージョン）】

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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International Bureau

(43) International Publication Date
21 March 2002 (21.03.2002)

PCT

(10) International Publication Number
WO 02/22874 A2

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Published:
— *without international search report and to be republished upon receipt of that report*

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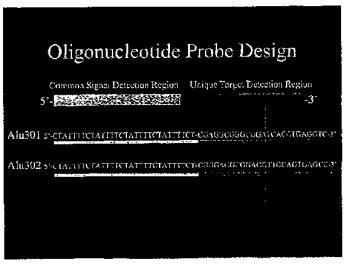
(54) Title: OLIGONUCLEOTIDE SEQUENCE FORMULA FOR LABELING OLIGONUCLEOTIDE PROBE AND PROTEINS FOR IN-SITU ANALYSIS






(L)60301681176

(57) Abstract: The present invention provides oligonucleotide probes and oligonucleotide probe collections and protein labeling for detecting or localizing a plurality nucleic acid target genes or antigens within a cell or tissue sample. Specifically, the provides collections of oligonucleotide probe for use in *in situ* hybridization analyses in which each probe has a label-domain with the sequence formulas of $(CTATTT)_n$, $(AAAATAG)_n$ or $(TTTTATC)_n$ or $(GATAAAA)_n$ in which all cases "n" would equal 1 or greater. The present invention provides collections or "cocktails" of oligonucleotide probes for detecting or localizing specific nucleic acid target genes within a cell or tissue sample. The cocktails are useful for detecting the following: the Kappa gene (SEQ ID NOS: 1-16 inclusive); the Lamba gene (SEQ ID NOS: 501-509, 511-513, and 515); the CMV (cytomegalovirus) gene (SEQ ID NOS: 221-241 inclusive); EBER (Epstein-Barr RNA) gene (SEQ IN NOS: 51-54 inclusive); Alu (SEQ IN NOS: 55-56); PolyA (SEQ ID NO: 57); and the detection tail (SEQ ID NO: 330).

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## BACKGROUND OF THE INVENTION

### 1. Field of the Invention

This invention relates to oligonucleotide probes and collections of oligonucleotide probes for detecting or localizing nucleic acid genes targets witin a cell or tissue sample. In particular, the invention relates to collections of oligoprobes.

### 2. Background of the Invention

*In situ* analysis includes in situ hybridization and immunohistochemistry. *In situ* hybridization (ISH) employs labeled DNA or RNA probe molecules that are anti-sense to a target gene sequence or transcript to detect or localize targeted nucleic acid target genes within a cell or tissue sample. ISH has proven to be a useful tool in a number of biomedical fields, including developmental biology, cell biology, and molecular biology. ISH has been used, for example, to diagnose genetic disorders, map genes, study gene expression, and localize sites of target gene expression.

Typically, ISH is performed by exposing a cell or tissue sample immobilized on a glass slide to a labeled nucleic acid probe which is capable of specifically hybridizing to a given target gene in the cell or tissue sample (*In Situ Hybridization: Medical Applications* (G. R. Coulton and J. de Belleroche, eds., Kluwer Academic Publishers, 1992); *In Situ Hybridization: In Neurobiology; Advances in Methodology* (J. H. Eberwine, K. L. Valentino, and J. D. Barchas, eds., Oxford University Press, 1994); *In Situ Hybridization: A Practical Approach* (D. G. Wilkinson, ed., Oxford University Press, 1992)). The hybridization of labeled probe molecules to nucleic acids in the cell or tissue sample can then be detected using, for example, radioactive-based direct detection methods, fluorescence-based direct detection methods, or indirect detection methods based on the binding of a fluorescence-labeled protein binding to a hapten such as BrdU, digoxigenin-labeled or biotin-labeled nucleotides incoporated into probes. Hapten-based methods have been further extended to include those molecules to be bonded by binding protein-enzyme conjugates such as antibody-enzyme-conjugates and colorimetric based detection chemistry. In addition, several target genes can be simulanteously analyzed by exposing a cell or tissue sample to a plurality of nucleic acid probes that have been labeled with a plurality of different nucleic

acid tags. For example, a plurality of nucleic acid probes can be labeled with a plurality of fluorescent compounds having different emission wavelengths, thereby permitting simultaneous multicolored analysis to be performed in a single step on a single target cell or tissue sample.

A significant problem associated with incorporation of labeled nucleotides into oligonucleotide probes is that the conjugation moieties that are attached to the nucleotide usually interfere with the formation of Watson-Crick base pairing, thus negatively affecting the hybridization of the probe to its target. The has been seen with use of label attached via N4-substitued cytosine nucleotides, because of steric hinderance and the expected shift to the less reactive state of a secondary amine (as seen with N4 labled cytosine), as compared to the natural G-C bond formed with an unsubstituted cytosine (a primary amine). Any small change or interference with G-C bonding in a small oligonucleotide (25 to 50 bases) can reduce the ability of these oligos to hybridize with the intended targeted sequence.

There remains a need in the art to develop suitable probes designs for incorporating labeled nucleotides in oligonucleotide probes. We demonstrate that a few artificial seqeunces are viable alternatives for probe labeling and also work both singly and in complex oligonucleotide probe mixtures for detecting or localizing nucleic acid target genes within a cell or tissue sample. The development of such generic seqeunces and labeling strategy for probe collections has wide application in the medical, genetic, and molecular biological arts.

This interference due to labeling chemistry and hybridization stringency and kinetics is solved herein by designing the oligo to have at least two distinct functional domains, one domain or sequence to be gene specific and involved in the base pair formation, and the second domain to be an artificial, non-specific sequence (in reference to the sample's genome) comprised of spacing nucleotides and the labeled nucleotide. These elements are positioned so that these label-nucleotides are more accessible as haptens for binding proteins (immunoglobulin or avidin(s)) and thus do not interfere with Watson-Crick base pairing in the gene-specific domain.

## SUMMARY OF THE INVENTION

The present invention provides a novel strategy to incorporate label into oligonucleotide probes and labeled oligonucleotide probe collections for detecting or

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localizing nucleic acid target genes within a cell or tissue sample. In particular, the invention relates to non-gene-specific sequences using sequence formulas for making repetitive polymers of such sequences which can be incorporated into collections of oligonucleotide probes for use in *in situ* hybridization analyses. In addition, using labeled synthetic oligonucleotide polymers, based on sequence formulas, when conjugated to binding proteins, i.e. immunglobulins, is a very effective and controlled process for labeling such proteins used in immunohistochemical analysis. The present invention provides collections or "cocktails" of oligonucleotide probes for detecting or localizing specific nucleic acid target genes within a cell or tissue sample. The cocktails are useful for detecting the following: the Kappa gene (SEQ ID NOS: 1-16 inclusive); the Lamba gene (SEQ ID NOS: 501-509, 511-513, and 515); the CMV (cytomegalovirus) gene (SEQ ID NOS: 221-241 inclusive); EBER (Epstein-Barr early RNA) gene (SEQ ID NOS: 51-54 inclusive); Alu (SEQ ID NOS: 55-56); PolyA (SEQ ID NO: 57); and the detection tail (SEQ ID NO:330).

The invention is directed to an oligonucleotide label-domain comprising the sequence $(CTATTTT)_n$ and its complement $(AAAATAG)_n$ wherein "n" is at least 1.

The invention is also directed to an oligonucleotide probe having at least two distinct functional domains, a first domain comprising the label-domain of claim 2, and a second domain comprising a gene-specific target sequence.

The invention is also directed to a probeset for detecting Kappa immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 401 through 416, inclusive.

The invention is also directed to a probeset for detecting Lambda immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 501 through 509, 511-513, and 515.

The invention is also directed to A probeset for detecting cytomegalovirus (CMV) immediate early RNA and /or corresponding mRNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 221 through 241

The invention is also directed to a probeset for detecting Epstein Barr virus (EBV) early RNA, RNA 1 and RNA 2, (EBER) wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 51 through 54.

The invention is also directed to a probeset for detecting Human Alu repetitive sattelite genomic DNA sequences wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 301 and 302.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a generic probe structure of of the two-domain probe design. This is the oligonucleotide design used for the probes in the gene specific cocktails described in the following examples. Each probe is composed of two domains: a 5' labeling domain and a 3' target gene target gene-specific domain. The labeling domain consists of this specific sequence (CTATTTT)n, wherein each cytosine may be labeled with a fluorophore or a cytosine-hapten conjugate, the hapten being fluorescein in this embodiment. This illustration specifically shows nucleic acid sequences for the 301 (SEQ ID NO: 55) and 302 (SEQ ID NO: 56) probes, each of which possesses target gene gene-specific domains corresponding to human repetitive Alu sequences and labeling domains having a fluorescein hapten.

Figure 2 illustrates the results obtained for *in situ* hybridization (ISH) analysis of human skin tissue using a probe comprising the labeling domain (330 probe; SEQ ID NO: 58). The absence of a detectable signal indicates that the sequence formula, (CTATTTT)n, of the labeling domain common to the oligonucleotides used in these ISH examples is non-specific, and non-reactive in its ability to form Watson-Crick base pairing with human nucleic acid sequences because it does not hybridize.

Figure 3 illustrates the results obtained for ISH analysis of human skin tissue using a probe comprising the labeling domain and a poly d(T) target gene-specific domain (320 probe; SEQ ID NO: 57). The presence of a detectable signal localized to the cytoplasm

indicates that this probe is capable of specifically hybridizing to polyadenylated region of messenger RNA.

Figures 4A-4B illustrate the results obtained for ISH analysis of human skin tissue using the 320 probe, wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The decrease in detectable signal in (B) indicates that this probe specifically hybridizes to polyadenylated region common to messenger RNA. .

Figures 5A-5B illustrate the results obtained for ISH analysis of human spleen tissue using the 320 probe, wherein the hybridization and stringency wash were performed at room temperature (A), or at 37°C (B). This result illustrates that the intenstity of color is related to the stringency of hybridization conditions, with the more intense color indicating less stringent conditons.

Figure 6 illustrates the results obtained for ISH analysis of the human Raji cell line using the 320 probe. This shows that this probe design also is functional with embedded cell lines as well as embedded tissue.

Figure 7 illustrates the results obtained for ISH analysis of the human Raji cell line using a probe collection consisting of the 301 and 302 probes.

Figure 8 illustrates the results obtained for ISH analysis of the human HT cell line using a probe collection consisting of the 301 and 302 probes.

Figure 9 illustrates the results obtained for ISH analysis of a rat cell line using a probe collection consisting of the 301 and 302 probes. The absence of a detectable signal indicates that this probe collection is specific for human nucleic acid sequences.

Figure 10 illustrates the results obtained for ISH analysis of an Epstein-Barr virus (EBV)-negative human HT cell line using a probe possessing a target gene-specific domain corresponding to EBV EBER nuclear RNA [SEQ ID NO: 51 through SEQ ID NO: 54?].

Figure 11 illustrates the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figure 12 illustrates the results obtained for ISH analysis of human tonsil tissue using a probe collection consisting of probes possessing target gene-specific domains

corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figures 13A-13B illustrate the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54, wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The decrease in detectable signal in (B) indicates that this probe specifically hybridizes to human EBER 1 and EBER 2 nuclear RNA.

Figure 14 illustrates the results obtained for ISH analysis of kappa light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain mRNA [ SEQ ID NO:15 ].

Figure 15 illustrates the results obtained for ISH analysis of lymphoma tissues using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin kappa light chain mRNA [ SEQ ID NOS: 2 - 4, SEQ ID NOS:7-12, SEQ ID NOS: 14, 15]. The lymphoma tissue in (A) over expresses the kappa light chain and the tissue in (B) over expresses the lambda light chain. The absence of a detectable signal in (B) indicates that the kappa light chain probe collection is specific to kappa light chain mRNA.

Figure 16 illustrates the results obtained for ISH analysis of lambda light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain variable region mRNA [ SEQ ID NOS:19 through 29].

Figure 17 illustrates the results obtained for ISH analysis of a lambda light chain-positive human RPMI 8226 cell line using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [ SEQ ID NOS:19 through 29].

Figures 18A-18B illustrate the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [ SEQ ID NOS:19 through 29]. The tissue in (A) over expresses the lambda light chain and the tissue in (B)

over expresses the kappa light chain. The absence of a detectable signal in (B) indicates that the lambda light chain probe collection is specific to human lambda light chain mRNA.

Figure 19 illustrates the results obtained for ISH analysis of cytomeglovirus (CMV)-positive human lung tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [ SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50]. [ CMV infected cell ]

Figure 20 illustrates the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has not been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early mRNA [ SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50].

Figures 21A-21B illustrate the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50. The tissue in (A) is shown at a magnification of 40X and the tissue in (B) is shown at a magnification of 20X.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

The present invention provides oligonucleotide probes and oligonucleotide probe collections for detecting or localizing nucleic acid target genes within a cell or tissue sample. In particular, the invention relates to collections of oligonucleotide probes for use in *in situ* hybridization analyses.

More specifically, this invention relates to the use of specific sequence formulas for nucleotide polymers or label-domains to attach a detectable moity ( a label) to oligonucleotide probes or proteins. The specific utility of these seqeuces or derivatives thereof, is the inert or non-reactive characteristic that does not hybridize to human DNA or RNA at a detectable level under standard stringency of hybridization conditions. These label-domains or polymers were demonstrated to be useful generic sequences for incorporation into oligonucleotide probes for detecting gene-specific sequences within cells or tissue samples in in situ hybridizaiton analyses. Additionally, this inert set of sequences

are useful for attaching a label to immunoglobulins or other proteins for detecting haptens and antigens in immunohistochemical analyses.

As used herein, the terms "probe" or "oligonucleotide probe" refers to a nucleic acid molecule used to detect a complementary nucleic acid target gene.

As used herein, the term "hybridization" refers to the process whereby complementary nucleic acid sequences join to form a double-stranded nucleic acid molecule. By labeling the target nucleic acid molecule with, for example, a radioactive or fluorescent tag, interactions between probe and target genes can be detected.

The oligonucleotide probes and oligonucleotide probes of the collections of the present invention are synthesized using conventional methods. *See e.g., Methods in Molecular Biology, Vol 20: Protocols for Oligonucleotides and Analogs* 165-89 (S. Agrawal, ed., 1993); *Oligonucleotides and Analogues: A Practical Approach* 87-108 (F. Eckstein, ed., 1991).

In a preferred embodiment of the present invention, oligonucleotide probes possess two distinct domains: a 5' (or labeling) domain and a 3' (or gene-specific target) domain (*See* Figure 1A). In more preferred embodiments of the present invention, the oligonucleotide probe possesses a labeling domain which consists of the sequence (CTATTTT)n. Other embodiments are also demonstrated herein, including a triple-domain embodiment having two terminal labeling domains, and a central gene-specific target domain. Specifically, SEQ ID NOS: 125-126 depict this labeling scheme. Yet a further preferred embodiment of a labeling domain is $TC(TTTTATC)_n$ or its complement. This sequence is predicted to be as unique as the (CTATTTT)n label-domain. The oligonucleotide probes of the present invention are labeled so that hybridization between said probes and target nucleic acids in a particular cell or tissue can be detected. Labels that are acceptable for use in *in situ* hybridization (ISH) analysis are known to those with skill in the art. Such labels permit interactions between probe and target genes to be detected using, for example, radioactive-based direct detection methods, fluorescence-based direct detection methods, digoxigenin-labeled or biotin-labeled probes coupled with fluorescence-based detection methods, or digoxigenin-labeled or biotin-labeled probes coupled with antibody-enzyme-based detection methods. In perferred embodiments of the present invention, oligonucleotide probes are labeled with fluorescein. In more preferred embodiments of the

present invention, the oligonucleotide probe possesses a labeling domain which consists of the sequence (CTATTTT)n, wherein the cytosine nucleotides may be labeled with a flurophore for direct detection, or a hapten for indirect detection. In either, the fluorescein-cytosine nucleotide conjugate and the fluorescein molecule is linked at the N4 position of cytosine through an OBEA linkage (*See* Mishra *et al.*, U.S. Patent No. 5,684,142, which is incorporated herein by reference). In a preferred embodiment, the density of fluorphore attached to the label-domain is at least 7 mole percent, preferably at least 10 mole percent, and most preferably at least 16 mole percent, when measured aginst the label-domain solely. For example, if probe 401 is considered (a 2-domain probe) it comprises a label-domain of 30 bases including a 3' terminal CT wherein the C is also labeled, the mole percent is 5/30 = 16.7 mole percent label. In the overall probe, the mole percent is 8.3.

In some embodiments of the present invention, several target genes are simulanteously analyzed by exposing a cell or tissue sample to a plurality of nucleic acid probes that have been labeled with a plurality of different nucleic acid tags. For example, a plurality of nucleic acid probes can be labeled with a plurality of fluorescent compounds having different emission wavelengths, thereby permitting simultaneous multicolored analysis to be performed in a single step on a single target cell or tissue sample.

The oligonucleotide probes and oligonucleotide probe collections of the present invention may be used in ISH analysis to detect or localize nucleic acid target genes within a cell or tissue sample. ISH may be performed as described, for example, in *In Situ Hybridization: Medical Applications* (G. R. Coulton and J. de Belleroche, eds., Kluwer Academic Publishers, 1992); *In Situ Hybridization: In Neurobiology; Advances in Methodology* (J. H. Eberwine, K. L. Valentino, and J. D. Barchas, eds., Oxford University Press, 1994); or *In Situ Hybridization: A Practical Approach* (D. G. Wilkinson, ed., Oxford University Press, 1992)).

The preferred embodiment of the probes and probe collections of the present invention are best understood by referring to Figures 1-21 and Examples 1-2. The Examples, which follow, are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

Probe Collection Preparation

Probe collections consisting of a plurality of oligonucleotide probes of 55 to 60 bases in length were designed as follows. In this Example, each oligonucleotide probe possessed two distinct domains: a 5' (or labeling) domain and a 3' (or target gene-specific) domain (*See* Figure 1).

In this embodiment, the labeling domain consists of the sequence (CTATTTT)n, wherein the cytosine nucleotide represents a fluorescein-cytosine nucleotide conjugate and the fluorescein molecule is linked at the N4 position of cytosine through an OBEA linkage.

The target gene-specific domain consists of a 25-30 base sequence that is complementary to a specific nucleic acid target gene. Oligonucleotide probes were designed to possess target gene-specific domains corresponding to the human immunoglobulin kappa light chain variable region (*See* Table 1; oligonucleotide probes 401-416), the human immunoglobulin lambda light chain variable region (oligonucleotide probes 501-515), human cytomegalovirus (CMV) sequences (oligonucleotide probes 221-241), human Esptein-Barr virus (EBV) EBER (Epstein-Barr early RNA) sequences (oligonucleotide probes 100A2, 100C2, 100A1, and 100B1), human repetitive Alu sequences (oligonucleotide probes 301 and 302), and poly d(T) (oligonucleotide probe 320).

EXAMPLE 2

Label-domain design: Alu repetitive sequence probe

Four probes all against the Alu human repetative sequence were used to evaluate label-domain design. The probes numbered 301 (SEQ ID NO: 55), 301A (SEQ ID NO:116), 301A2/2 (SEQ ID NO: 121), and 301A3/2 (SEQ ID NO: 122) are shown in Table 1.

The four probes were evaluated at the concentrations of 100, 75, 50, and 25 ng/ml per mL of probe in the reaction, respectively. This hybridization analysis was done manually, using standard protocols. The target, paraffin-embedded cell line MBA MD 468 (Oncor INFORM™ Her-2/neu Control Slides, Cat. No. S8100, Level 1, available from Ventana Medical Systems, Inc., Tucson, AZ) was the target sample and was processed by removing paraffin by standard xylene methods. The tissue was subjected to Ventana's. Protease 1 for 12 minutes at 50 degrees C as a 1:2 dilution with Ventana's APK buffer. The hybridization reaction was accomplished with the addition of probe diluent as 100 ul probe (25%

formamide, 5% dextran sulfate, 2X SSC, 1% Triton) to a residual 100 ul volume of 2XSSC/Triton X-100. The slide was heated to 85 degrees C for 5 minutes and then incubated for 1 hr at 37 degrees C. Standard SSC washes followed for removing excess probe. The hybrids were detected with an antibody against FITC. The mouse antibody was detected colormetrically using Ventana Enhanced Alkaline Phosphatase Blue Detection (cat# 760-061). Unless otherwise indicated, all reagents were obtained from Ventana Medical Systems, Inc., Tucson, AZ. The results were observed by colormetric detection using brightfield microscopy.

The results of these experiments were that signal intensity was a function of the total number of fluorescein hapten conjugated to the probe and signal was of the specific label-domain design. The greater the number of fluoresceins per probe molecule, the greater the signal observed. Comparison of design and placement of haptens on the probe showed that this was not a factor in signal intensity. The two probes that contained five fluoresceins, (301A3/2 (SEQ ID NO: 122) and 301 (SEQ ID NO:55) both yielded equivalent signal. These two probes yielded greater signal that seen for 301A2/2, a probe with a split label-domain design with four fluoresceins. The probe 301A2/2 yielded a signal greater than probe 301A a probe with a single label-domain design at the 5' end and with three fluoresceins.

**EXAMPLE 3**

**Label Domain Design: EBER probes**

This experiment compared two label-domain designs and sequences to determine whether greater spacing between the fluorescein haptens improves the production of signal during probe detection steps during *in situ* hybridization analysis.

The tissue used was an EBV-infected human spleen tissue fixed in neutral buffered formalin paraffin embedded section of 4-micron thickness placed on silane plus glass microscope slides. The tissue sections were deparaffinized on a Ventana DISCOVERY™ machine, followed by a 6-min digestion with Ventana's Protease 1, at a temperature of 37 C. The probe was dissolved in hybridization buffer diluent at a concentration of 50 ng/mL as a 100 ul applied to an equal volume of 2 X SSC/ Triton X-100 residual volume left on the slide after prepared by the Ventana Medical Systems, Inc. automated ISH staining system, Discovery. The probe diluent-mixed with the residual volume on slide for 6 min at 37 C,

then the solution was heated to 85C and held there for a total of 10 min. The slide was then taken to a 37C temperature and held at that temperature for 1 hour. All of these aqueous reactions on the slide were all done under a film of LIQUID COVERSLIP™, to prevent evaporative loss of water during processing. Each slide after hybridization was washed 3 times with 2X SSC/Triton solution, with a 6 min incubation between each wash, the slide volume being approximately 300 ul (+/- 10% vol). The hybrids were detected with an antibody against FITC. The mouse antibody was detected colormetrically using Ventana Enhanced Alkaline Phosphatase Blue Detection (cat# 760-061).

The two oligonucleotide probes used for this study probe 100A1 (SEQ ID NO: 53) and 1002A32 (SEQ ID NO: 120). The two differences between these probes were the label-domain seqence and structure. The probe 100A1 label domain was 5' to gene target domain, contained 5 fluoresceins attached to cytosine residues via the OBEA linker, with the sequence formula of $(CTATTTT)_4CT$ (SEQ ID NO: 58). The label domain of the oligo probe 1002A32, was similiar, (SEQ ID NO:125). Besides the different sequence the primary difference was that the fluoresceine labeled cytosines were spaced 10 bases apart compared to the oligo 100A1 the cytosine spacing was closer at 7 bases apart. The result of this comparison as deduced by H score analysis were that these oligonucleotide were equivalent as to the amount of signal generated on the slide. The data was that for 100A2, for the 368 cells analysed in a total of 3 fields the H score was 106, and for probe 1002A32 for the 345 cell analysed in three field the H score was 109. The H score is a spectrographic analysis done with micrscope that factors into the score background to signal ratio on the tissue section to yield a relative comparison of total target specific signal on the slide. (See reference Giroud, F. Perrin C, and Simony Lafontaine, J.; Quantitative Immunocytochemistry and Immunohistochemistry. Third Conference of the European Society for Analytical Cellular Pathology, 1994; and AutoCyte Quic Immuno User's Manual, 1998, document number PA-029, Co AutoCyte Inc. Burlington NC 2721). The histograms and the score sheet indicated that each oligo were equally efficient in yielding a colormetric signal. This indicates that the position of the label domain can be either 3 prime or 5 prime to the gene target sequence or the gene target sequence can be positioned between two label domains.

**EXAMPLE 4**

***In Situ* Hybridization**

The probe collections prepared in Example 1 were first diluted in a solution consisting of 20% dextran sulfate (wt/vol), 50% formamide (vol/vol), 2X SSC, 10 mM Tris-HCl, 5 mM EDTA, and 0.05% Brij-35, at a final pH of 7.3. Probe collections were then mixed with an equal volume of a solution consisting of 2X SSC and 0.05% Triton X-100.

Samples for ISH analysis were prepared by cutting formalin-fixed and paraffin-embedded cells or tissue samples into 4 μm sections and placing the sections onto a glass slide. Subsequent processing and ISH of samples was carried out in an automated device, such as the DISCOVERY™ Automated ISH/IHC Stainer (Ventana Medical Systems, Inc., Tucson, AZ) described in co-owned and co-pending U.S. Patent App. Serial Nos. 60/076,198 and 09/259,240, both incorporated herein by reference. . To remove paraffin from the samples, the slides were immersed in an aqueous solution, heated for approximately 20 minutes, and then rinsed. The automated deparaffinization procedure is more fully described in U.S. Serial No. 60/099,018, 09/259,240 both incorporated herein by reference. The samples were then treated with protease and the slides were heated to 85°C (for hybridization to RNA target genes) or 90-95°C (for hybridization to DNA target genes) for 4 to 10 minutes.

Hybridization reactions were typically performed in a hybridization buffer consisting of 10% dextran sulfate (wt/vol), 25% formamide (vol/vol), 2X SSC, 5 mM Tris, 2.5 mM EDTA, 0.025% Brij-35, 0.25% Triton X-100, and between 25 to 125 ng/mL of each individual probe molecule. ISH reactions were performed at between 37°C to 54°C. For ISH using the probe collections described in Example 1, hybridization reactions were optimally carried out for 1 hr at 47°C (except for the poly d(T) probe, wherein the hybridization reaction was optimally carried out at 37°C for 1 hr).

The hybridization of fluorescein-labeled probe molecules to a particular target gene in the sample was detected by using a sequential series of binding proteins, i.e., secondary antibody detection. However, it is equally possible to use detect detection when visualizing the bound probes. In secondary detection, first, an anti-fluorescein mouse monoclonal antibody directed against the fluorescein-labeled probe molecule was added to the sample. Next, a biotin-labeled polyclonal goat antibody directed against the mouse antibody was added to the sample. Finally, hybridization reactions were colormetrically detected using a 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) substrate This technique, termed "secondary antibody detection," is routine for one of skill in the art.

Primary and secondary antibodies are available from numerous suppliers, including Ventana Medical Systems, Tucson, AZ, which are optimized for use on the Ventana autostaining systems (ES®, NexES®, DISCOVERY™, and BENCHMARK™).

Figures 2-21 illustrate the results obtained for *in situ* hybridization analysis of various cell lines or tissue samples using the probes disclosed and claimed herein having the structural motif illustrated in Figure 1 or probe collections consisting of such probes.

Figure 1 illustrates a generic probe structure of of the two-domain probe design. This is the oligonucleotide design used for the probes in the gene specific cocktails described in the following examples. Each probe is composed of two domains: a 5' labeling domain and a 3' target gene target gene-specific domain. The labeling domain consists of this specific sequence (CTATTTT)n, wherein the cytosine nucleotide is a cytosine-hapten conjugate, the hapten being fluorescein in this embodiment. This illustration specifically shows nucleic acid sequences for the 301 (SEQ ID NO: 55) and 302 (SEQ ID NO: 56) probes, each of which possesses target gene gene-specific domains corresponding to human repetitive Alu sequences and labeling domains having a fluorescein hapten.

Figure 2 illustrates the results obtained for *in situ* hybridization (ISH) analysis of human skin tissue using a probe comprising the labeling domain (330 probe; SEQ ID NO: 58). The absence of a detectable signal indicates that the sequence formula, (CTATTTT)n, of the labeling domain common to the oligonucleotides used in these ISH examples is non-specific, and non-reactive in its ability to form Watson-Crick base pairing with human nucleic acid sequences because it does not hybridize.

Figure 3 illustrates the results obtained for ISH analysis of human skin tissue using a probe comprising the labeling domain and a poly d(T) target gene-specific domain (320 probe; SEQ ID NO: 57). The presence of a detectable signal localized to the cytoplasm indicates that this probe is capable of specifically hybridizing to polyadenylated region of messenger RNA.

Figures 4A-4B illustrate the results obtained for ISH analysis of human skin tissue using the 320 probe, wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The decrease in detectable signal in (B) indicates that this probe specifically hybridizes to polyadenylated region common to messenger RNA. .

Figures 5A-5B illustrate the results obtained for ISH analysis of human spleen tissue using the 320 probe, wherein the hybridization and stringency wash were performed at room temperature (A), or at 37°C (B). This result illustrates that the intenstity of color is related to the stringency of hybridization conditions, with the more intense color indicating less stringent conditons.

Figure 6 illustrates the results obtained for ISH analysis of the human Raji cell line using the 320 probe. This shows that this probe design also is functional with embedded cell lines as well as embedded tissue.

Figure 7 illustrates the results obtained for ISH analysis of the human Raji cell line using a probe collection consisting of the 301 and 302 probes.

Figure 8 illustrates the results obtained for ISH analysis of the human HT cell line using a probe collection consisting of the 301 and 302 probes.

Figure 9 illustrates the results obtained for ISH analysis of a rat cell line using a probe collection consisting of the 301 and 302 probes. The absence of a detectable signal indicates that this probe collection is specific for human nucleic acid sequences.

Figure 10 illustrates the results obtained for ISH analysis of an Epstein-Barr virus (EBV)-negative human HT cell line using a probe possessing a target gene-specific domain corresponding to EBV EBER nuclear RNA [SEQ ID NO: 51 through SEQ ID NO: 54].

Figure 11 illustrates the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figure 12 illustrates the results obtained for ISH analysis of human tonsil tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54].

Figures 13A-13B illustrate the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to EBV EBER 1 and 2 nuclear RNA [SEQ ID NO:51 through SEQ ID NO:54], wherein the tissue sample was not treated with ribonuclease A prior to *in situ* hybridization (A), or was treated with ribonuclease A prior to *in situ* hybridization (B). The

decrease in detectable signal in (B) indicates that this probe specifically hybridizes to human EBER 1 and EBER 2 nuclear RNA .

Figure 14 illustrates the results obtained for ISH analysis of kappa light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NO:15].

Figure 15 illustrates the results obtained for ISH analysis of lymphoma tissues using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin kappa light chain mRNA [SEQ ID NOS: 2 - 4, SEQ ID NOS:7-12, SEQ ID NOS: 14, 15]. The lymphoma tissue in (A) over expresses the kappa light chain and the tissue in (B) over expresses the lambda light chain. The absence of a detectable signal in (B) indicates that the kappa light chain probe collection is specific to kappa light chain mRNA.

Figure 16 illustrates the results obtained for ISH analysis of lambda light chain-positive human tonsil tissue using a probe possessing a target gene-specific domain corresponding to human immunoglobulin lambda light chain variable region mRNA [SEQ ID NOS:19 through 29].

Figure 17 illustrates the results obtained for ISH analysis of a lambda light chain-positive human RPMI 8226 cell line using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NOS:19 through 29].

Figures 18A-18B illustrate the results obtained for ISH analysis of human spleen tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to human immunoglobulin lambda light chain mRNA [SEQ ID NOS:19 through 29]. The tissue in (A) over expresses the lambda light chain and the tissue in (B) over expresses the kappa light chain. The absence of a detectable signal in (B) indicates that the lambda light chain probe collection is specific to human lambda light chain mRNA.

Figure 19 illustrates the results obtained for ISH analysis of cytomeglovirus (CMV)-positive human lung tissue using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50]. Arrow indicates CMV infected cell.

Figure 20 illustrates the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has not been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50].

Figures 21A-21B illustrate the results obtained for ISH analysis of a rat 9G cell line in which the expression of CMV immediate early RNA has been induced by cyclohexamide using a probe collection consisting of probes possessing target gene-specific domains corresponding to CMV immediate early RNA [SEQ ID NOS:30-32, SEQ ID NOS: 34-35, SEQ ID NO: 38, SEQ ID NO: 50] expression of the CMV immediate early RNA with cyclohexamide. The tissue in (A) is shown at a magnification of 40X and the tissue in (B) is shown at a magnification of 20X.

**Table 1**

| Probe ID | Sequence | SEQ ID |
|---|---|---|
| 401 | 5'-CTATTTCTATTTTCTATTTTCTATTTTCT CCAGAGTAGCAGGAGCCCCAGGAGCTGAGC-3' | 1 |
| 402 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGATGGAGACTGGGTCAACTGGATGTCACA-3' | 2 |
| 403 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GCAAGCGATGGTGACTCTGTCTCCTACAGC-3' | 3 |
| 404 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TCTGTCCCAGATCCACTGCCACTGAACCTT-3' | 4 |
| 405 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GCAGCCACAGTTCGCTTCATCTGCACCTTG-3' | 5 |
| 406 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TTTCAACTGCTCATCAGATGGCGGGAAGAT-3' | 6 |
| 407 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AAGTTATTCAGCAGGCACACAACAGAGGCA-3' | 7 |
| 408 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGCGTTATCCACGTTCCACTGTACTTTGGC-3' | 8 |
| 409 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TAGGTGCTGTCCTTGCTGTCCTGCTCTGTG-3' | 9 |
| 410 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GTAGTCTGCTTTGCTCAGCGTCAGGGTGCT-3' | 10 |
| 411 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GATGGGTGACTTCGCAGGCGTAGACTTTGT-3' | 11 |
| 412 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CTCTCCCCTGTTGAAGCTCTTTGTGACGGG-3' | 12 |
| 413 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TGGAACTGAGGAGCAGGTGGGGGCACTTCT-3' | 13 |
| 414 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GAAAAAGGGTCAGAGGCCAAAGGATGGGAG-3' | 14 |
| 415 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT AGATGAGCTGGAGGACCGCAATAGGGGTAG-3' | 15 |

| | | | |
|---|---|---|---|
| 416 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GCATAATTAAAGCCAAGGAGGAGGAGGGGG-3' | 16 |
| 501 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CCTGAGTGAGGAGGGTGAGGAGCAGCAGAG-3' | 17 |
| 502 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AGACCCAGACACGGAGGCAGGCTGAGTCAG-3' | 18 |
| 503 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TGTTGGTTCCAGTGCAGGAGATGGTGATCG-3' | 19 |
| 504 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TAAATCATGATTTTGGGGGCTTTGCCTGGG-3' | 20 |
| 505 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TGTTGCCAGACTTGGAGCCAGAGAAGCGAT-3' | 21 |
| 506 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AATAATCAGCCTCGTCCTCAGCCTGGAGCC-3' | 22 |
| 507 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GGTCCCTCCGCCGAAAACCACAGTGTAACT-3' | 23 |
| 508 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TTATGAGACACACCAGTGTGGCCTTGTTGG-3' | 24 |
| 509 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CTGCTCAGGCGTCAGGCTCAGATAGCTGCT-3' | 25 |
| 511 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ATGCGTGACCTGGCAGCTGTAGCTTCTGTG-3' | 26 |
| 512 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ATTCTGTAGGGGCCACTGTCTTCTCCACGG-3' | 27 |
| 513 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | CCTCCCCTGGGATCCTGCAGCTCTAGTCTC-3' | 28 |
| 515 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TGAGGGTTTATTGAGTGCAGGGAGAAGGGC-3' | 29 |
| 221 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GGAGGTCAAAACAGCGTGGATGGCG-3' | 30 |
| 222 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GAGGCTGGATCGGTCCCGGTGTCTT-3' | 31 |
| 223 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AATCCGCGTTCCAATGCACCGTTCC-3' | 32 |
| 224 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TAAAAACTGCGGGCACTGGGGACGG-3' | 33 |
| 225 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ACCCGAGATTCGCGTGGAGATCCCA-3' | 34 |
| 226 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | GAGCAAGGAGCTGCCGAGCGACCAT-3' | 35 |
| 227 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | ACACTGGTGGTGGTGGGCATCGTGC-3' | 36 |
| 228 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | TTCCAAATGCGTCAGCGGTGCAAGC-3' | 37 |
| 229 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | AGCTGCCTGCATCTTCTTCTGCCGC-3' | 38 |

20

| | | |
|---|---|---|
| 238 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TCTCAGAGGATCGGCCCCCAGAATG-3' | 47 |
| 239 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCTCATCTGACTCCTCGGCGATGGC-3' | 48 |
| 240 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGGGTACAGGGGACTCTGGGGGTGA-3' | 49 |
| 241 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGGTGGGTGCTCTTGCCTCCAGAGG-3' | 50 |
| 100A2 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GACCTCGGGTCGGTAGCACCGCACT-3' | 51 |
| 100C2 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGAAGCCTCTCTTCCTCCCCCGG-3' | 52 |
| 100A1 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CCACAGACACCGTCCTCACCACCCG-3' | 53 |
| 100B1 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT GGCTACAGCCACACACGTCTCCTCC-3' | 54 |
| 301 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGAGGCGGGCGGATCACCTGAGGTC-3' | 55 |
| 302 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 56 |
| 320 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT TTTTTTTTTTTTTTTTTTTTTTTTT-3' | 57 |
| 301A | 5'-CTATTTTCTATTTTCTTTT CGAGGCGGGCGGATCACCTGAGGTC-3' | 116 |
| 302C | 5'-CTATTTTCTATTTTCTTTT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 117 |
| 302A4 | 5'-CTATTTATACTTTATATTTCATATTTTATCT CGGGAGGCGGAGGTTGCAGTGAGCC-3' | 118 |
| 302A3/2 | 5'-CTATTTTATATTTATATTTCT CGGGAGGCGGAGGTTGCAGTGAGCC ACTATTTTATACTT-3 | 119 |
| 1002A32 | 5'-CTATTTTATACTTTATATTTCT GACCTCGGGTCGGTAGCACCGCAC TACTATTTTATACTT-3' | 120 |
| 301A2/2 | 5'-CTATTTTTCTT CGAGGCGGGCGGATCACCTGAGGTC TTCTTTTTATCTT-3 | 121 |
| 301A3/2 | 5'-CTATTTTATACTTTATATTTCT CGAGGCGGGCGGATCACCTGAGGTC ACTATTTTATACTT-3' | 122 |

Table 2

| Probe ID | Sequence | SEQ ID |
|---|---|---|
| | 5'-CTATTTTTCTATTTTTCTTTT | 123 |
| | 5'-CTATTTTATACTTTATATTTCATATTTTATCT | 124 |
| 330 | 5'-CTATTTTCTATTTTCTATTTTCTATTTTCT | 58 |
| | 5'-CTATTTTATACTTTATATTTCT.....ACTATTTTATACTT-3 | 125 |
| | 5'-CTATTTTTCTT.....TTCTTTTTATCTT-3 | 126 |

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

WE CLAIM:

1. An oligonucleotide label-domain comprising the sequence $(CTATTTT)_n$ and its complement $(AAAATAG)_n$ wherein "n" is at least 1.

2. The oligonucleotide label-domain of claim 1 detectably labeled with a reporter molecule, or a hapten molecule.

3. The oligonucleotide label-domain of claim 2 wherein the hapten is flurorescein linked to the N4 nitrogen of cytosine through an OBEA linker.

4. The oligonucleotide label-domain of claim 1 wherein the reporter molecule is a fluorophore.

5. The oligonucleotide label-domain of claim 1 wherein the fluorophore is present at a density of greater than 7 mole percent.

6. The oligonucleotide label-domain of claim 1 wherein the label-domain has the sequence $TC(TTTTATC)_n$ ( or its complementary formula).

7. The oligonucleotide label-domain of claim 1 wherein the sequence is SEQ ID NO: 58.

8. The oligonucleotide label-domain of claim 2 wherein at least 7 mole pecent of the cytosines are linked to a detectable moiety by an OBEA linker.

9. An oligonucleotide probe having at least two distinct functional domains, a first domain comprising the label-domain of claim 2, and a second domain comprising a gene-specific target sequence.

10. The oligonucleotide probe of claim 9 wherein the label-domain is located at the 5' end of the oligonucleotide probe, and the gene-specific target sequence being 3' to the label-domain.

11. The oligonucleotide probe of claim 9 wherein the label-domain is located at the 3' end of the oligonucleotide probe, and the gene-specific target sequence is 5' to the label-domain.

12. An oligonucleotide probe having three distinct functional domains, a first domain comprising the label-domain of claim 2, a second domain comprising a gene-specific target sequence, and a third domain comprising another label-domain, wherein said second domain is located between said first and third domains.

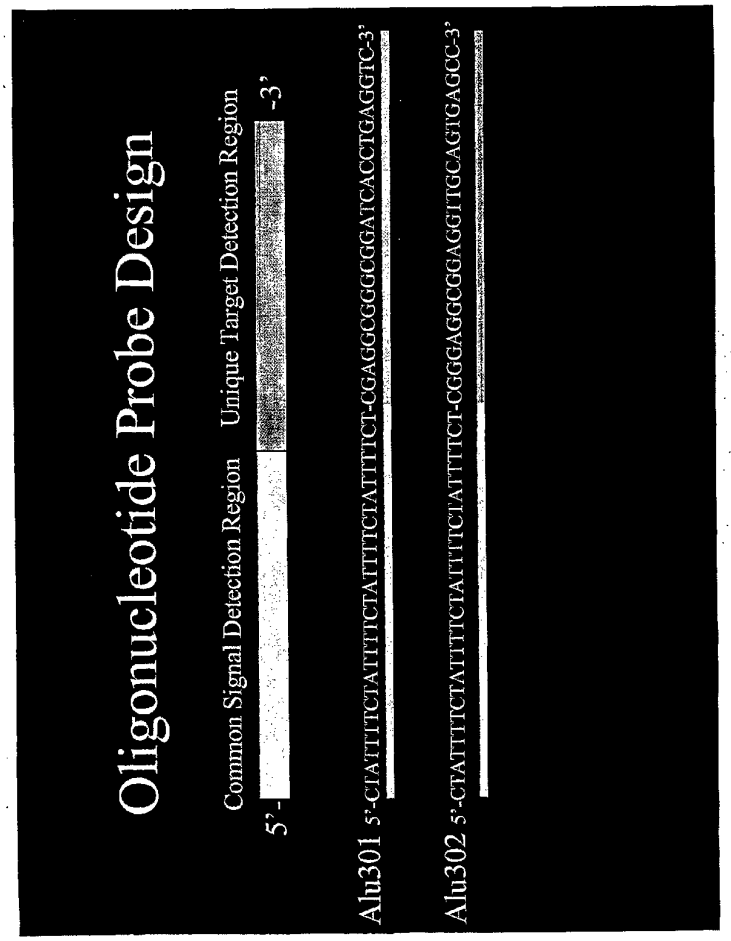
13. A probeset for detecting Kappa immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 401 through 416, inclusive.

14. A probeset for detecting Lambda immunoglobulin light chain mRNA or corresponding hetereonuclear RNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 501 through 509, 511-513, and 515.

15. A probeset for detecting cytomegalovirus (CMV) immediate early RNA and /or corresponding mRNA wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 221 through 241

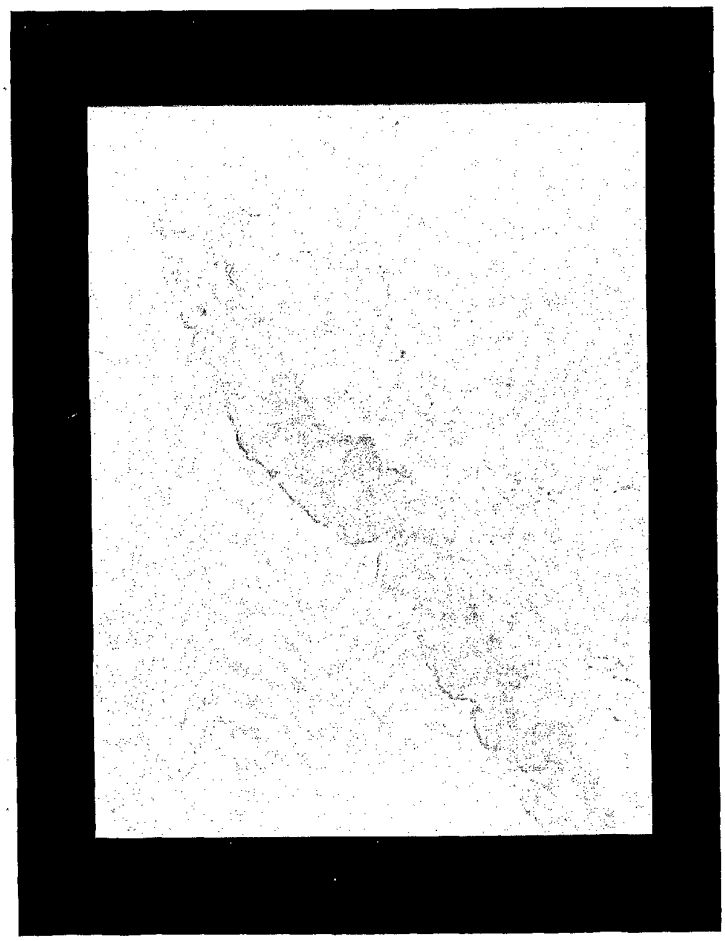
16. A probeset for detecting Epstein Barr virus (EBV) early RNA, RNA 1 and RNA 2, (EBER) wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 51 through 54.

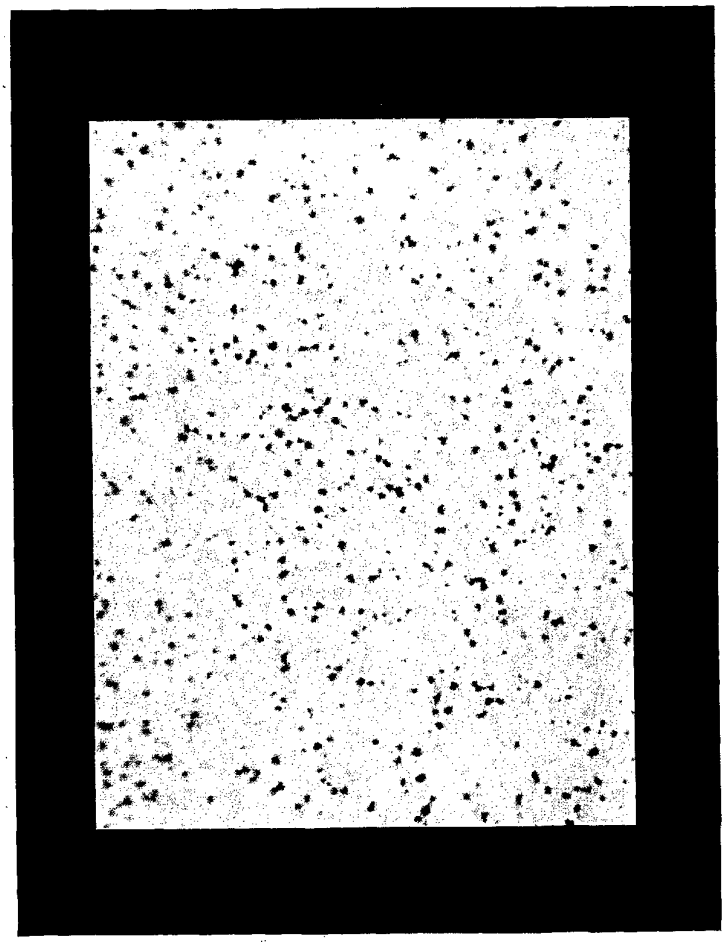
17. A probeset for detecting Human Alu repetitive sattelite genomic DNA sequences wherein the probes are selected from the group consisting essentially of SEQ ID NOS: 301 and 302.


Oligonucleotide Probe Design
Common Signal Detection Region
Unique Target Detection Region
5'-
-3'
Alu301 5'-CTATTTCTATTTTCTATTTTCT-CGAGGCGGCGGATCACCTGAGGTC-3'
Alu302 5'-CTATTTTCTATTTTCTATTTTCT-CGGGAGGCGGAGGTTGCAGTGAGCC-3'

25







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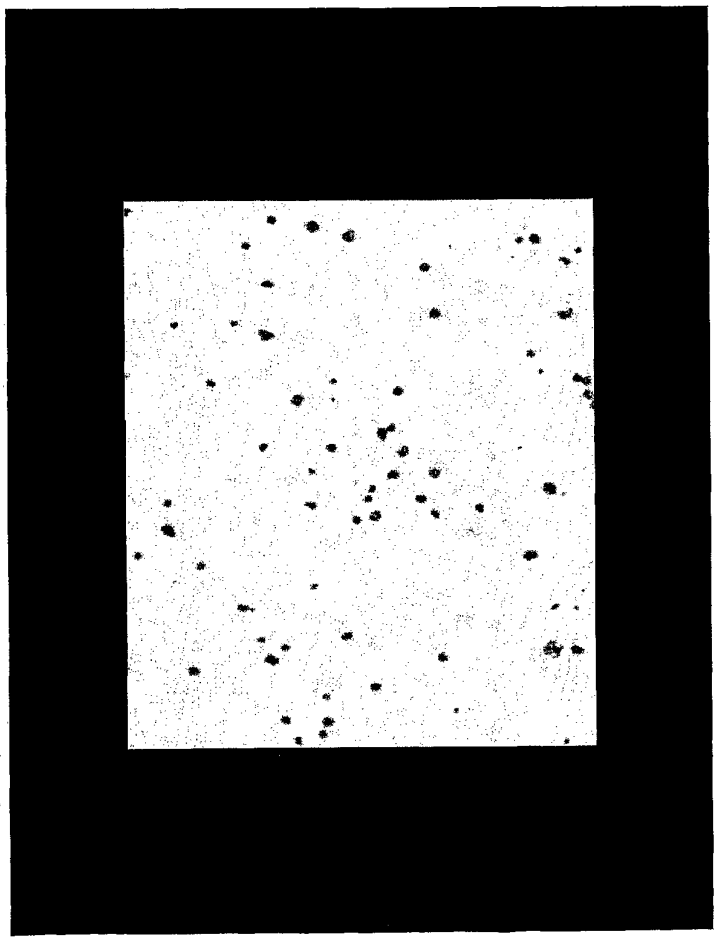

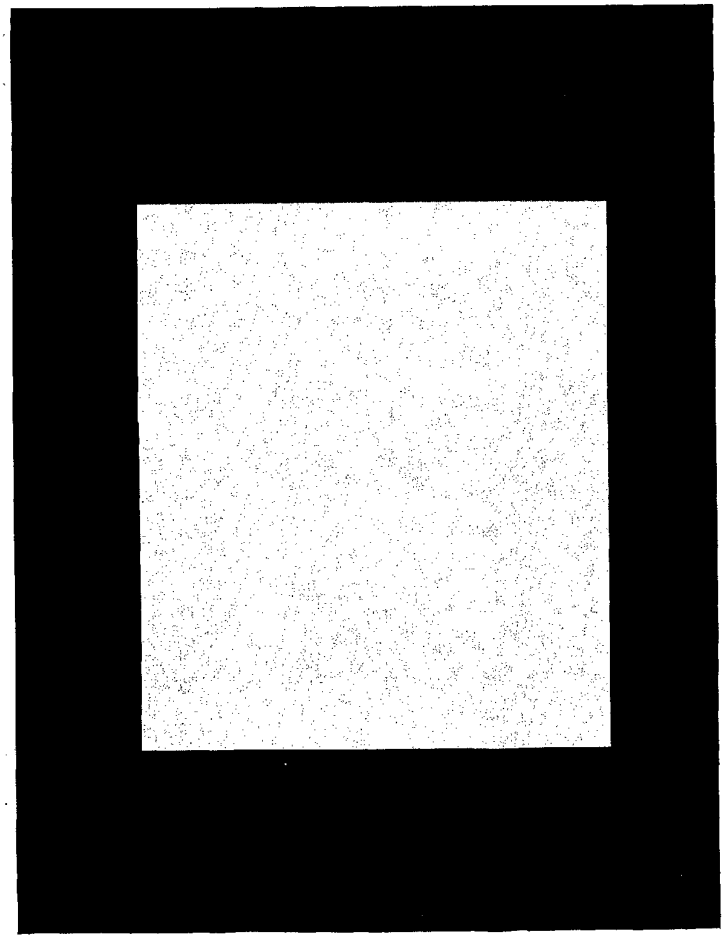

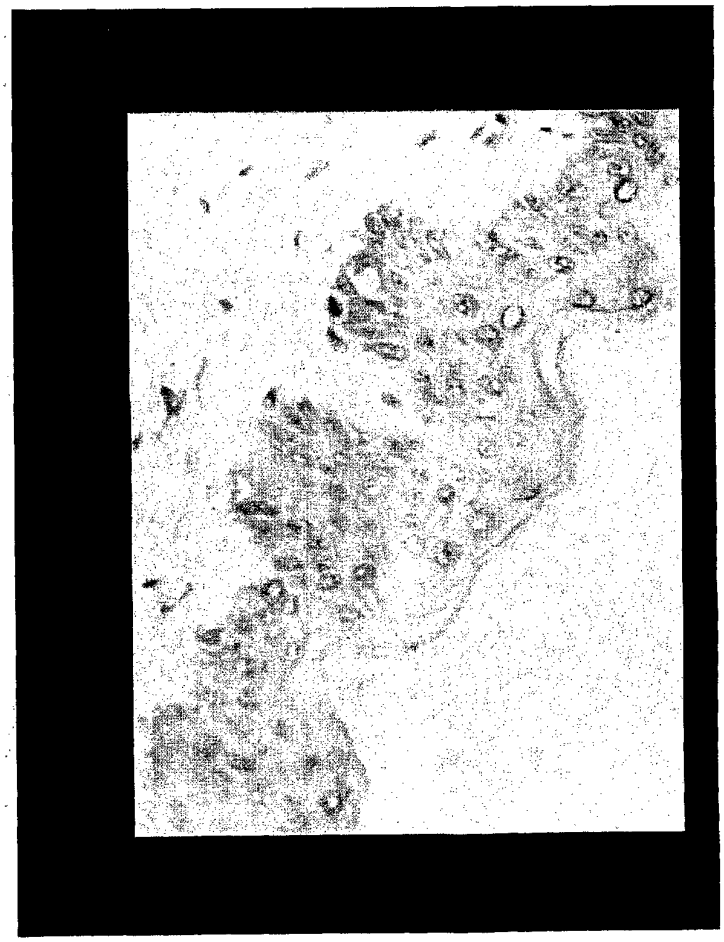

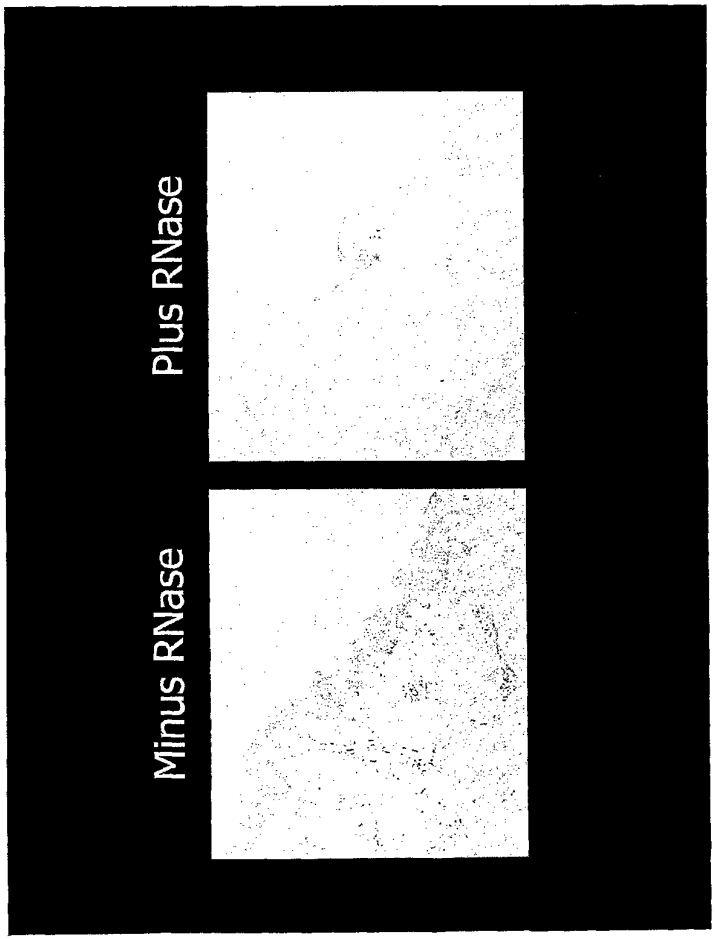
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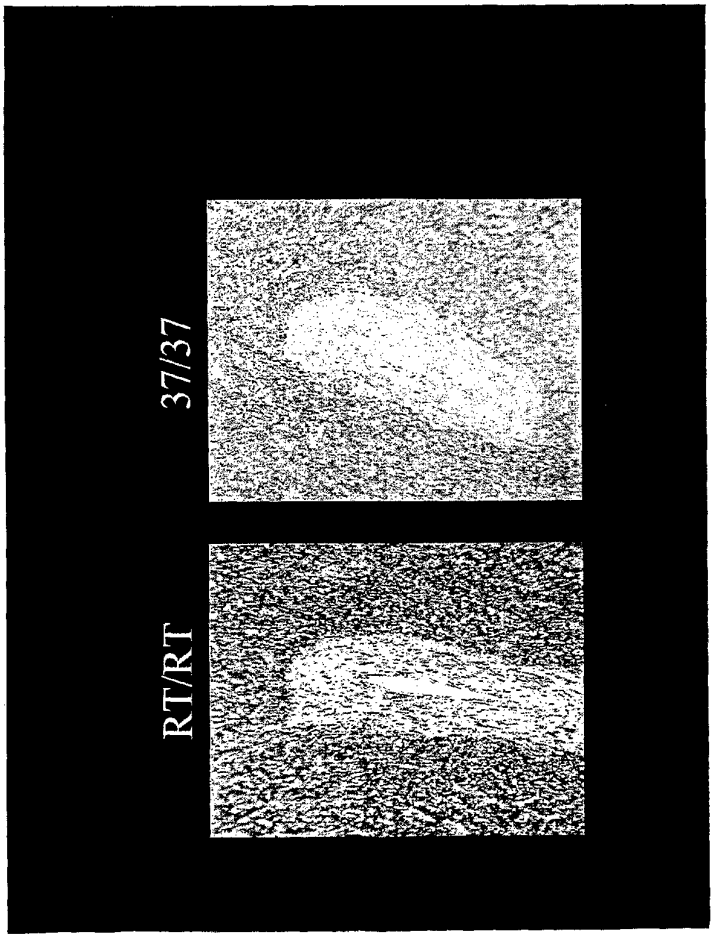
Minus RNase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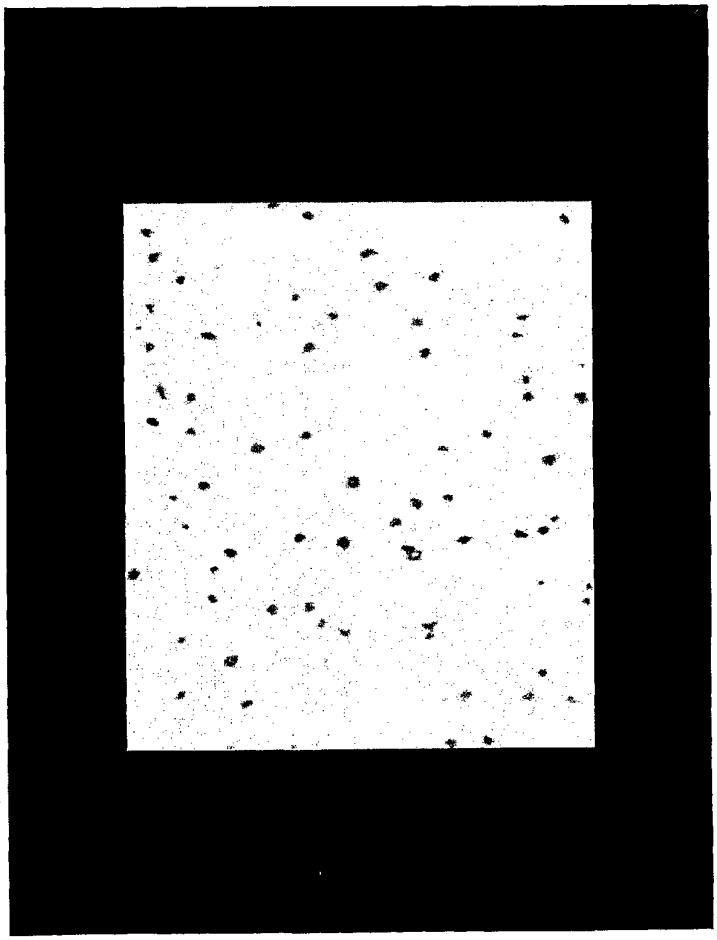
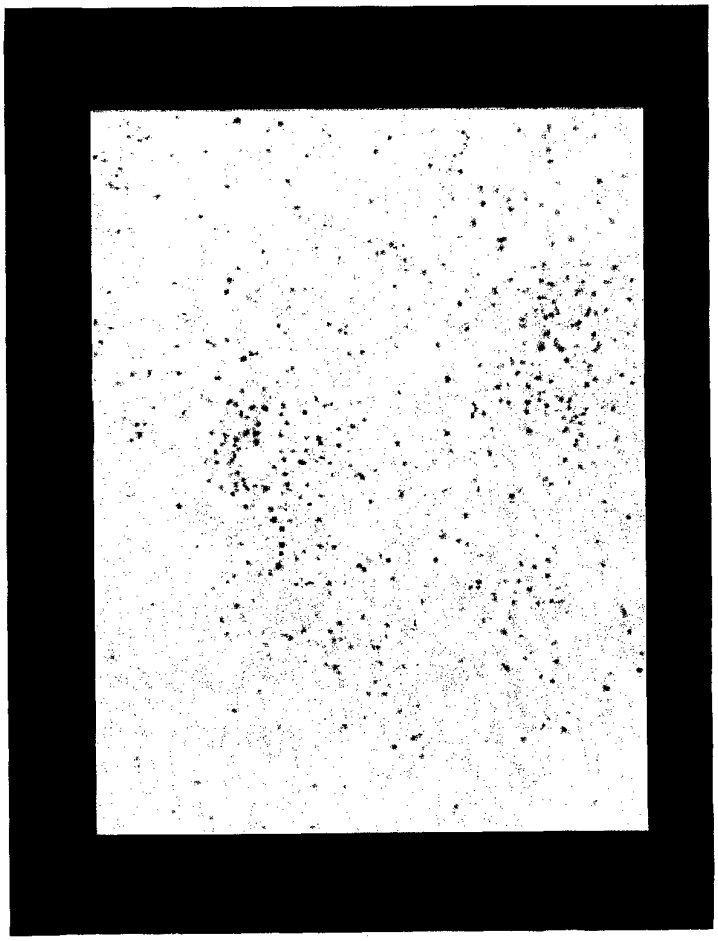
RT/RT





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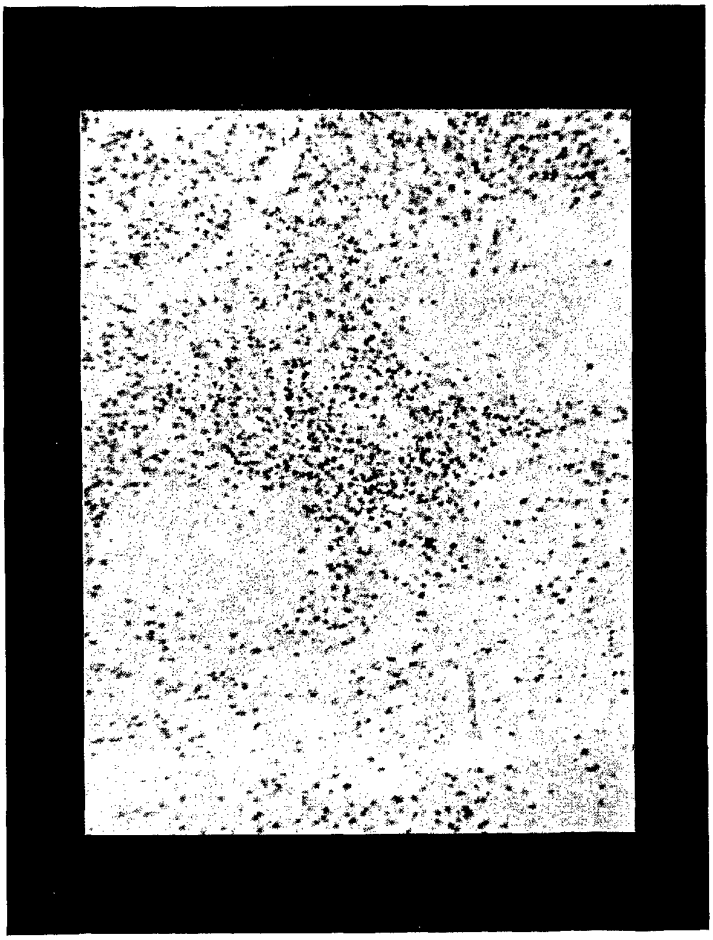

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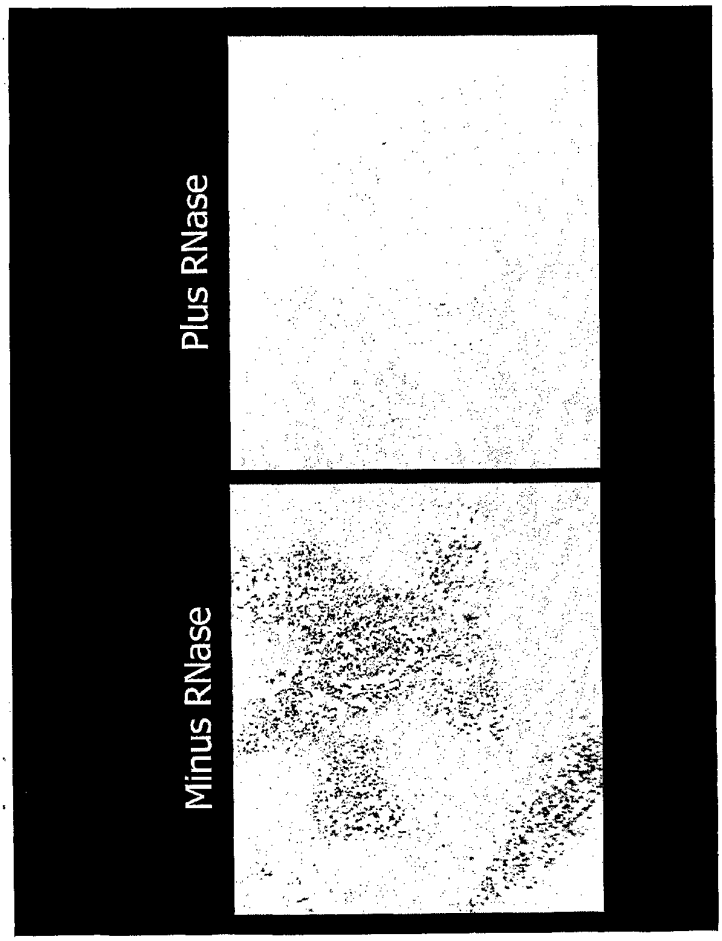

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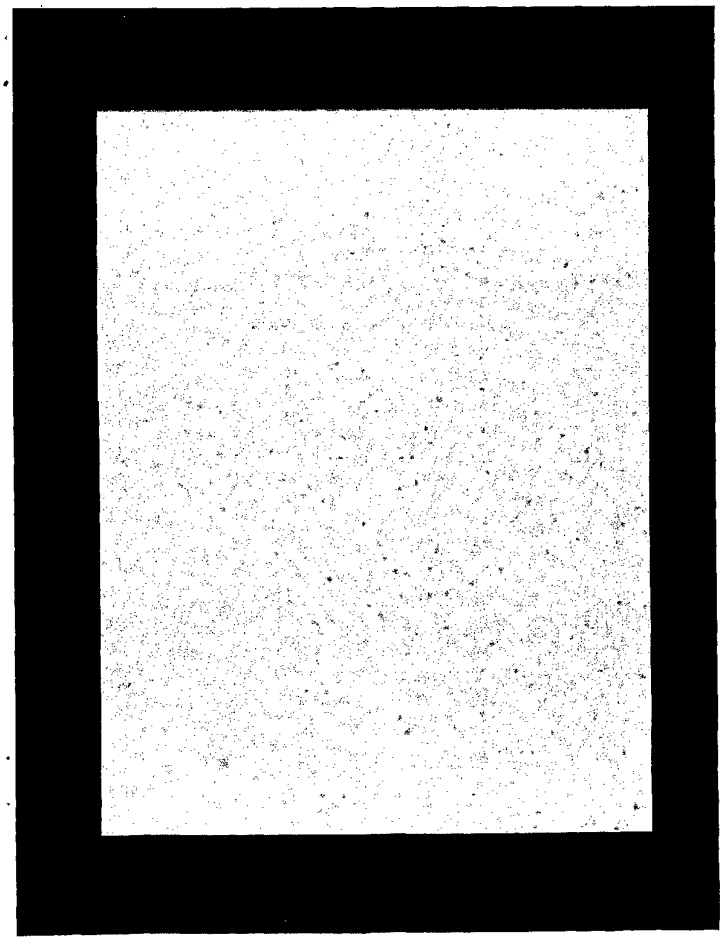

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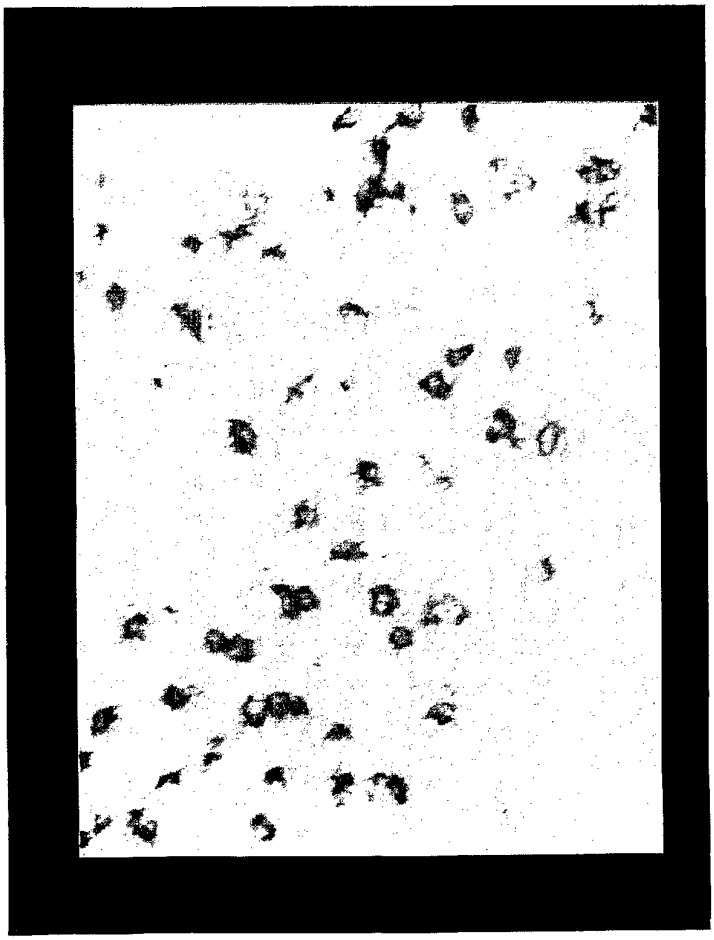

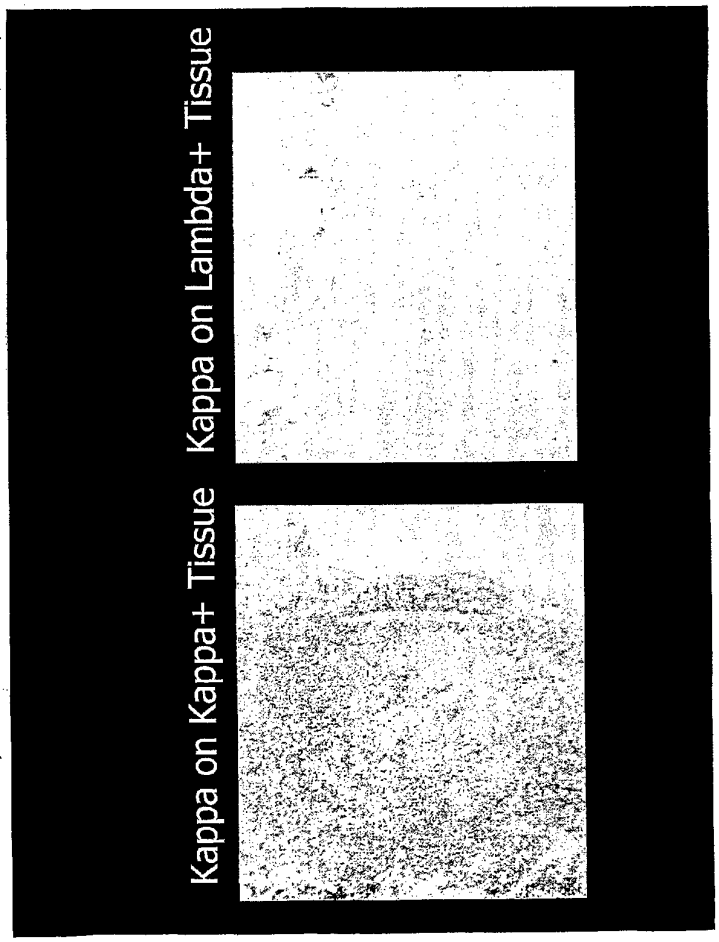
Kappa on Kappa+ Tissue
Kappa on Lambda+ Tissue

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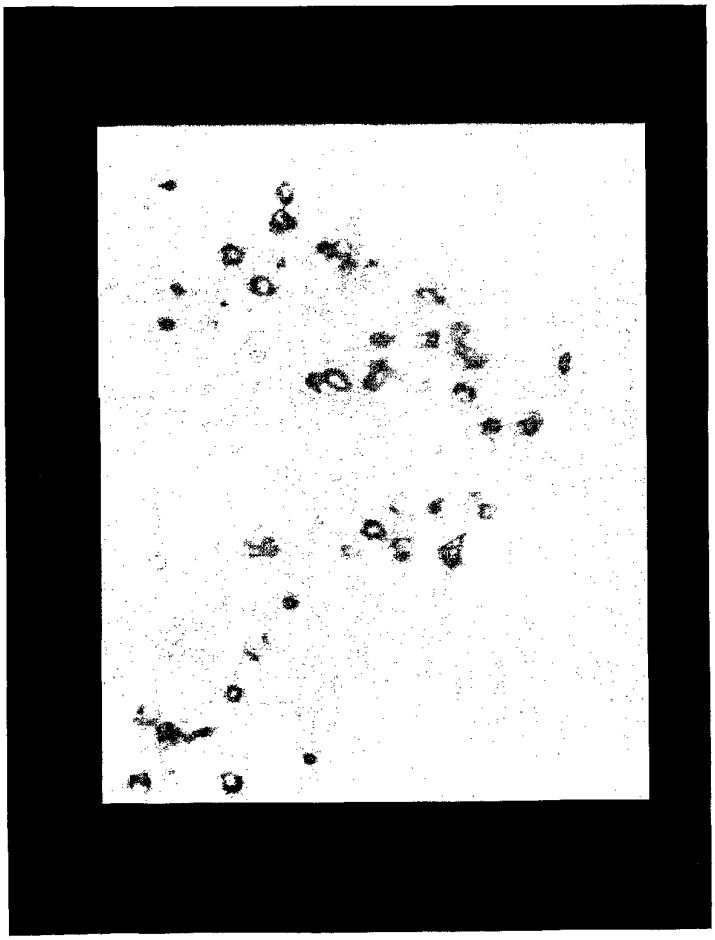

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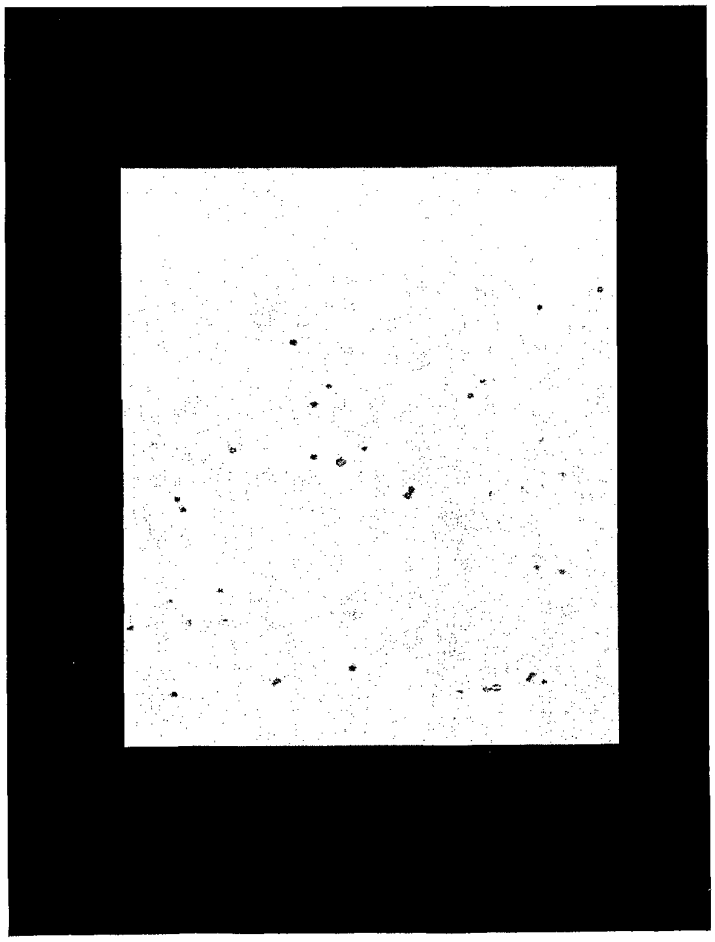

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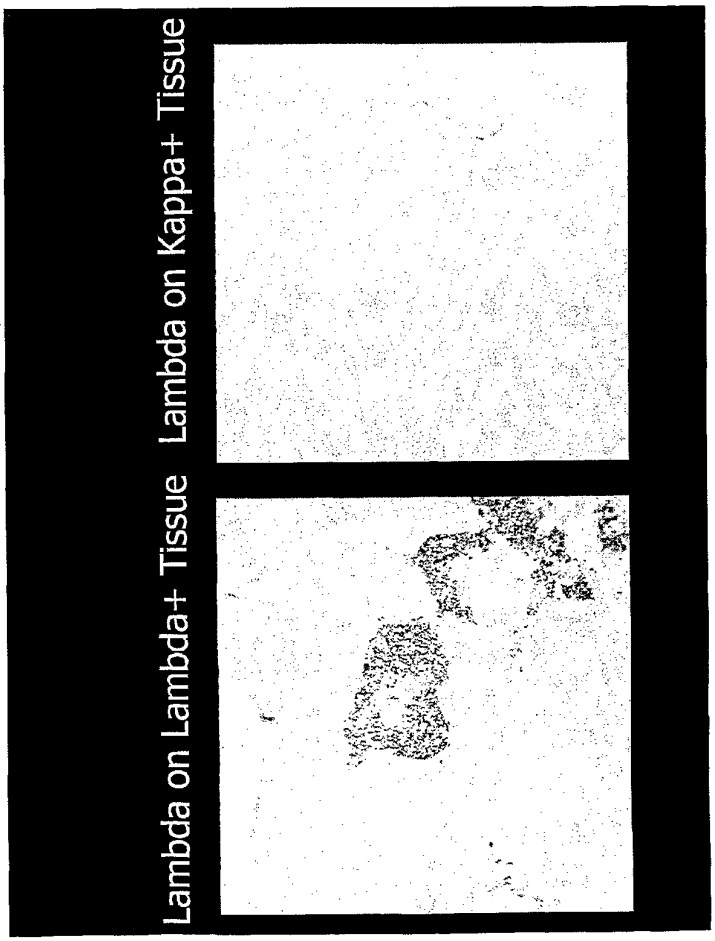

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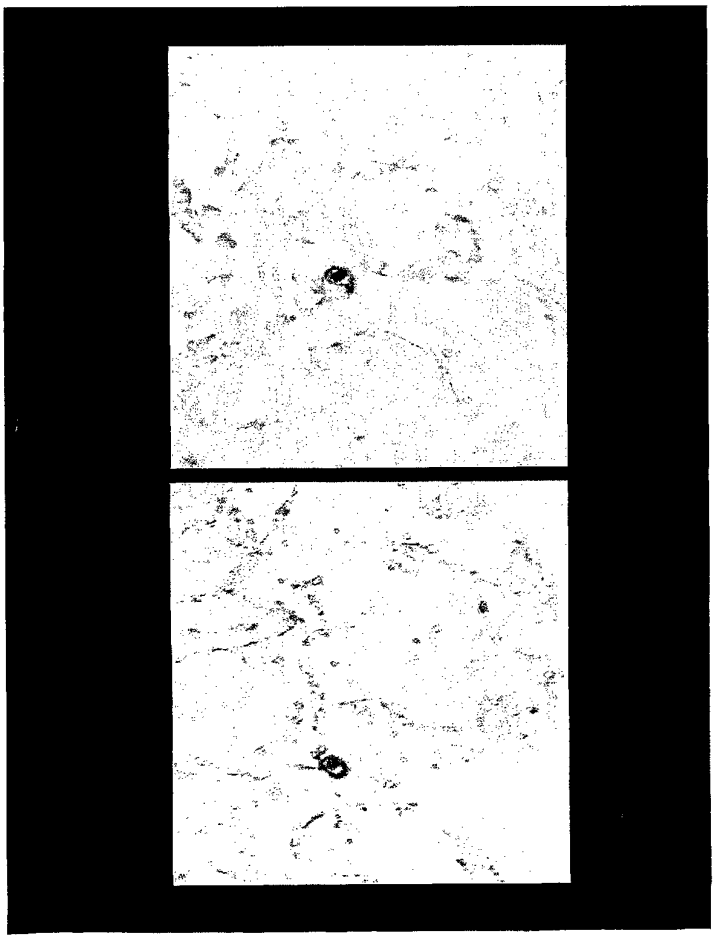

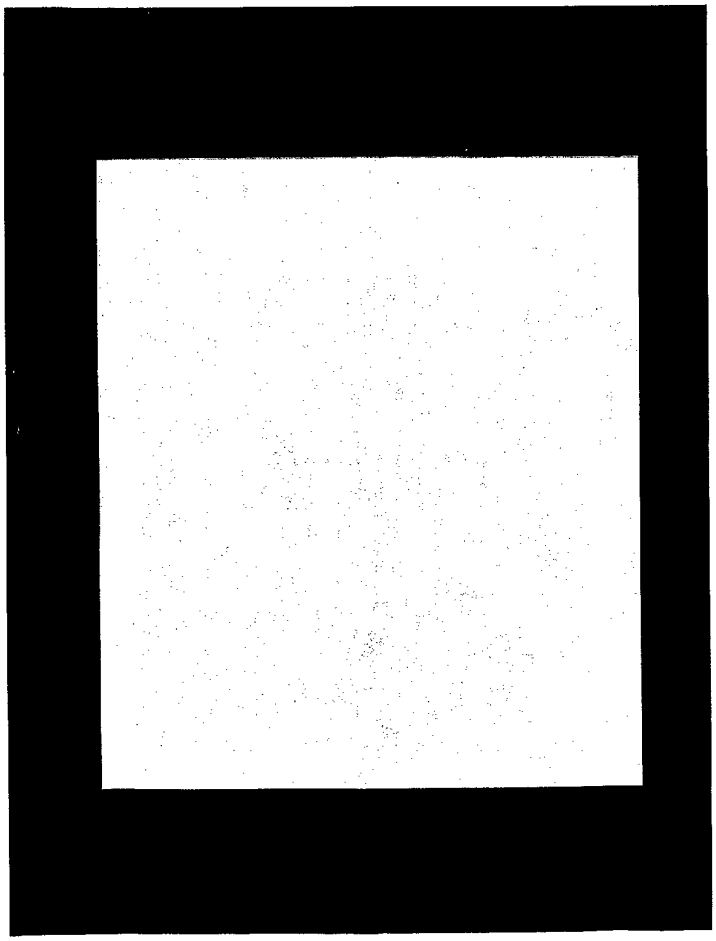

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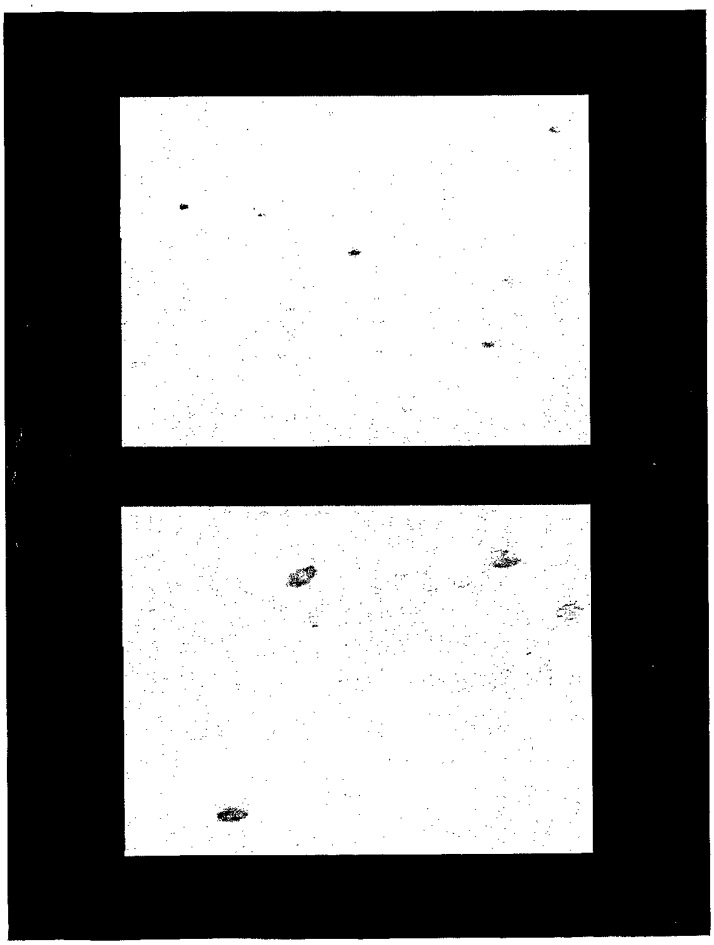

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<210> 32
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<213> Artificial Sequence

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<223> Description of Artificial Sequence:

52

Oligonucleotide probe

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<210> 33
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<213> Artificial Sequence

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<223> Description of Artificial Sequence:
Oligonucleotide probe

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<210> 34
<211> 55
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<223> Description of Artificial Sequence:
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<210> 35
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<400> 35
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<210> 36
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<400> 36
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<210> 37
<211> 55
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<400> 37
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<210> 38
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<210> 39
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Oligonucleotide probe

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<210> 40
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<210> 41
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<210> 42

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<211> 55
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<210> 43
<211> 55
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<210> 44
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<210> 45
<211> 55
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Oligonucleotide probe

<400> 45
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<210> 46
<211> 55
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<210> 47
<211> 55
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<223> Description of Artificial Sequence:
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<210> 48
<211> 55
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<210> 49
<211> 55
<212> DNA
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 49
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<210> 50
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<210> 51
<211> 55
<212> DNA
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<220>
<223> Description of Artificial Sequence:

Oligonucleotide probe

<400> 51
ctattttcta ttttctattt tctattttct gacctcgggt cggtagcacc gcact 55

<210> 52
<211> 55
<212> DNA
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<220>
<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 52
ctattttcta ttttctattt tctattttct ggaagcctct cttctcctcc cccgg 55

<210> 53
<211> 55
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<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 53
ctattttcta ttttctattt tctattttct ccacagacac cgtcctcacc acccg 55

<210> 54
<211> 56
<212> DNA
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<220>
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Oligonucleotide probe

<400> 54
ctattttcta ttttctattt tctattttct ggctacagcc acacacgtct cctccc 56

<210> 55
<211> 55
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<223> Description of Artificial Sequence:
Oligonucleotide probe

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ctattttcta ttttctattt tctattttct cgaggcgggc ggatcacctg aggtc 55

<210> 56
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<400> 56
ctatttttcta ttttctattt tctatttttct cgggaggcgg aggttgcagt gagcc 55

<210> 57
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<400> 57
ctatttttcta ttttctattt tctatttttct ttttttttttt ttttttttttt ttttttttttt 60

<210> 58
<211> 30
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<400> 58
ctatttttcta ttttctattt tctatttttct 30

<210> 59
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<400> 59
ccagagtagc aggagcccca ggagctgagc 30

<210> 60
<211> 30
<212> DNA
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<400> 60
ggatggagac tgggtcaact ggatgtcaca 30

<210> 61

<211> 30
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<223> Description of Artificial Sequence:
Oligonucleotide probe

<400> 61
gcaagcgatg gtgactctgt ctcctacagc 30

<210> 62
<211> 30
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tctgtcccag atccactgcc actgaacctt 30

<210> 63
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<400> 63
gcagccacag ttcgcttcat ctgcaccttg 30

<210> 64
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<400> 64
tttcaactgc tcatcagatg gcgggaagat 30

<210> 65
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<400> 65

aagttattca gcaggcacac aacagaggca 30

<210> 66
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<400> 66
ggcgttatcc accttccact gtactttggc 30

<210> 67
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taggtgctgt ccttgctgtc ctgctctgtg 30

<210> 68
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Oligonucleotide probe

<400> 68
gtagtctgct ttgctcagcg tcagggtgct 30

<210> 69
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gatgggtgac ttcgcaggcg tagactttgt 30

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tggaactgag gagcaggtgg gggcacttct 30

<210> 72
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gaaaaagggt cagaggccaa aggatgggag 30

<210> 73
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agatgagctg gaggaccgca ataggggtag 30

<210> 74
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gcataattaa agccaaggag gaggaggggg 30

<210> 75
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cctgagtgag gagggtgagg agcagcagag 30

<210> 76
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agacccagac acggaggcag gctgagtcag 30

<210> 77
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tgttggttcc agtgcaggag atggtgatcg 30

<210> 78
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<400> 78
taaatcatga ttttgggggc tttgcctggg 30

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tgttgccaga cttggagcca gagaagcgat 30

<210> 80

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<210> 81
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ggtccctccg ccgaaaacca cagtgtaact 30

<210> 82
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ttatgagaca caccagtgtg gccttgttgg 30

<210> 83
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<400> 83
ctgctcaggc gtcaggctca gatagctgct 30

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63

atgcgtgacc tggcagctgt agcttctgtg 30

<210> 85
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<400> 85
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<210> 86
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cctcccctgg gatcctgcag ctctagtctc 30

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tgagggttta ttgagtgcag ggagaagggc 30

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ggaggtcaaa acagcgtgga tggcg 25

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gaggctggat cggtcccggt gtctt 25

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aatccgcgtt ccaatgcacc gttcc 25

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taaaaactgc gggcactggg gacgg 25

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gagcaaggag ctgccgagcg accat 25

<210> 94
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acactggtgg tggtgggcat cgtgc 25

<210> 95
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ttccaaatgc gtcagcggtg caagc 25

<210> 96
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agctgcctgc atcttcttct gccgc 25

<210> 97
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ccctccaccg ttaacagcac cgcaa 25

<210> 98
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ttggtcacgg gtgtctcggg cctaa 25

<210> 99

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<210> 100
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tcggggttct cgttgcaatc ctcgg 25

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<210> 106
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<210> 107
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<400> 107
cgggtacagg ggactctggg ggtga 25

<210> 108
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<400> 108
gggtgggtgc tcttgcctcc agagg 25

<210> 109
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<400> 109
gacctcgggt cggtagcacc gcact 25

<210> 110
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<210> 111
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<400> 111
ccacagacac cgtcctcacc acccg 25

<210> 112
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ggctacagcc acacacgtct cctccc 26

<210> 113
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cgaggcgggc ggatcacctg aggtc 25

<210> 114
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cgggaggcgg aggttgcagt gagcc 25

<210> 115
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ttttttttt ttttttttt ttttttttt 30

<210> 116
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<400> 116
CTATTTTTCTATTTTTCTTTT CGAGGCGGGCGGATCACCTGAGGTC 46

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<210> 118

70

<211>
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CTATTTTATACTTTATATTTCATATTTTATCT CGGGAGGCGGAGGTTGCAGTGAGCC 57

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Oligonucleotide probe

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Oligonucleotide probe

<400> 121
CTATTTTTCTT CGAGGCGGGCGGATCACCTGAGGTC TTCTTTTTATCTT 49

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61

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<210> 124
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<400> 125
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<210> 126
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Oligonucleotide probe

<400> 126
CTATTTTTCTTNNNNNNNNNNNNNNNNNNNNNNNNNNNTTCTTTTTATCTT 49

【国際調査報告】

**INTERNATIONAL SEARCH REPORT**

International Application No
PCT/US 01/28014

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | DIRKS R W ET AL: "Methodologies for specific intron and exon RNA localization in cultured cells by haptenized and fluorochromized probes." JOURNAL OF CELL SCIENCE. ENGLAND APR 1993, vol. 104 ( Pt 4), April 1993 (1993-04), pages 1187-1197, XP002226881 ISSN: 0021-9533 | |

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 10 January 2003 | 13. 05. 03 |
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Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/28014

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.: 1-6,8-11
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-11

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-6,8-11

Present claims 1-6 and 8-11, relate to an extremely large number of possible compounds. Moreover, claim 1 lacks clarity: it is not clear whether the sequence (CTATTTT)n and the sequence (AAAATAG)n should be in the same strand or whether the claim intends to include double stranded molecules.

Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely oligonucleotides exactly identical to SEQ ID 58 (included in the subject-matter of claim 7).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-11

Any nucleic acid molecule at least containing CTATTTT and/or AAAATAG at least once. N.B. this inlcudes the subject-matter of claim 9 because the "second domain" is not defined and can be more or less any sequence.

2. Claim : 12

Any nucleic acid sequence containing either CTATTTT and/or AAAATAG twice, where these sequences flank another sequence.

3. Claims: 13-17

Groups 3-58: each individual SEQ ID as set out in claims 13-17.

フロントページの続き

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Ｆターム(参考) 4B024 AA20　CA01　CA09　CA11　HA14　HA19

【要約の続き】
－ＢａｒｒＲＮＡ）遺伝子（配列番号：５１－５４を含めて）；Ａｌｕ（配列番号：５５－５６）；ポリＡ（配列番号：５７）；および検出テール（配列番号：３３０）。

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|---|---|---|---|
| 专利名称(译) | <无法获取翻译> | | |
| 公开(公告)号 | JP2005503753A5 | 公开(公告)日 | 2008-12-04 |
| 申请号 | JP2002527314 | 申请日 | 2001-09-06 |
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| IPC分类号 | C12N15/09 G01N33/53 G01N33/532 G01N33/569 | | |
| CPC分类号 | C12Q1/6841 C12Q1/6811 C12Q1/6816 C12Q1/6876 C12Q1/6888 C12Q2600/158 C12Q2525/161 C12Q2525/151 C12Q2537/143 | | |
| FI分类号 | C12N15/00.ZNA.A G01N33/53.M G01N33/532.B G01N33/569.L | | |
| F-TERM分类号 | 4B024/AA20 4B024/CA01 4B024/CA09 4B024/CA11 4B024/HA14 4B024/HA19 | | |
| 优先权 | 60/233177 2000-09-15 US | | |
| 其他公开文献 | JP4986367B2<br>JP2005503753A | | |


摘要(译)

本发明提供寡核苷酸探针和寡核苷酸探针集合和蛋白质标记，用于检测或定位细胞或组织样品中的多个核酸靶基因或抗原。具体而言，本发明是，每个探针序列式（CTATTTT）名词，（AAAATAG）名词，或（TTTTATC）名词或（GATAAAA）名词 [其中，所有当“n”是等于或超过在1]一种用于在具有与标签域的情况下的原位杂交分析中使用寡核苷酸探针。本发明提供寡核苷酸探针的集合或“混合物”，用于检测或定位细胞或组织样品中的特定核酸靶基因。鸡尾酒是用于检测以下::卡帕基因（SEQ ID NO：1-16，其中包括）有用;拉姆达基因（SEQ ID NO：501-509,511-513，和515）; CMV（巨细胞病毒）基因（SEQ ID NO：221-241包括）; EBER（爱泼斯坦 - BarrRNA）基因（SEQ ID NO：51-54，含）;铝（SEQ ID NO：55-56）;聚一（SEQ ID NO：57 ）;和检测尾（SEQ ID NO：330）。