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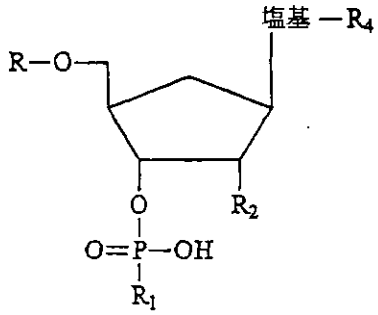
(54)【発明の名称】化学合成した核酸の純度を測定する方法と組成物

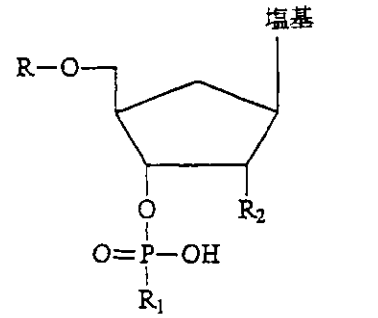
(57)【要約】
本願は、有機保護基が共有結合している合成オリゴマー（例えば、オリゴヌクレオチドまたはオリゴペプチド）に特異的に結合し、有機保護基が共有結合していない場合にはその合成オリゴマーに結合しない抗体を記載している。このような抗体を製造する方法および使用する方法と、このような抗体を製造する細胞と、このような抗体を用いたアッセイ手法に使用することができる固定オリゴマーを有する物品も開示されている。

【特許請求の範囲】
【請求項１】
有機保護基が共有結合している合成オリゴヌクレオチドに特異的に結合し、前記有機保護基が共有結合していない場合には前記合成オリゴヌクレオチドに結合しない抗体。
【請求項２】
前記オリゴヌクレオチドが３～２０のヌクレオチドからなり、前記ヌクレオチドの１つが、式（Ｉ）、
【化１】



(I)

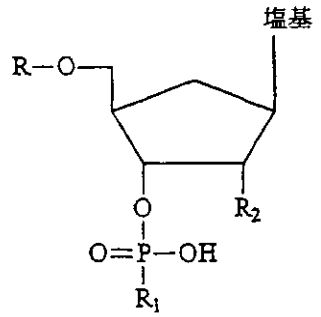
（式中、ＲはＨまたは保護基であるが、ただし、前記保護されている塩基が前記ヌクレオチドの５’末端ヌクレオチドではない場合には、Ｒは隣接ヌクレオチドとの共有結合であり、
R_1 はＨまたは保護基であるが、ただし、前記保護されている塩基が前記ヌクレオチドの３’末端ヌクレオチドでない場合には、R_1 は隣接ヌクレオチドとの共有結合であり、
R_2 はＨまたは－OR_3 であり、
R_3 はＨまたは保護基であり、
塩基はプリンまたはピリミジン塩基であり、
R_4 は前記塩基のアミノ基に結合した保護基であるが、
ただし、Ｒ、R_1、R_3 および R_4 の１つが保護基である場合には、Ｒ、R_1、R_3 および R_4 の残りは保護基でない）の保護されているヌクレオチドである、請求項１に記載の抗体。
【請求項３】
前記オリゴヌクレオチドが３～２０のヌクレオチドからなり、５’ヌクレオチドを有し、前記５’ヌクレオチドが、式（Ｉ）、
【化２】



(I)

（式中、Ｒは保護基であり、
R_1 は隣接ヌクレオチドとの共有結合であり、
R_2 は－Ｈまたは－ＯＨであり、
塩基はプリンまたはピリミジン塩基である）の保護されているヌクレオチドである、請求項１に記載の抗体。
【請求項４】
前記オリゴヌクレオチドが３～２０のヌクレオチドからなり、３’ヌクレオチドを有し、前記３’ヌクレオチドが、式（Ｉ）、

【化３】



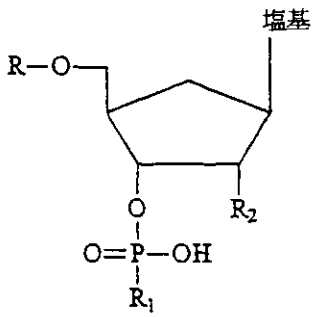
(I)

（式中、Ｒは隣接ヌクレオチドとの共有結合であり、
Ｒ１は保護基であり、
Ｒ２は－Ｈまたは－ＯＨであり、
塩基はプリンまたはピリミジン塩基である）の保護されているヌクレオチドである、請求項１に記載の抗体

【請求項５】

前記オリゴヌクレオチドが３～２０のヌクレオチドからなり、前記ヌクレオチドの１つが、式（Ｉ）、

【化４】



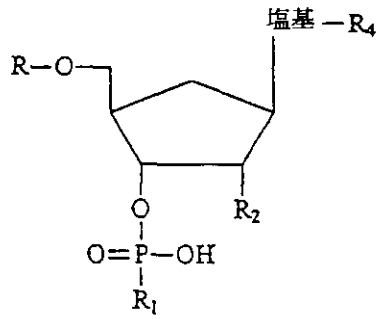
(I)

（式中、Ｒは隣接ヌクレオチドとの共有結合であり、
Ｒ１は隣接ヌクレオチドとの共有結合であり、
Ｒ２は－ＯＲ３であり、
Ｒ３は保護基であり、
塩基はプリンまたはピリミジン塩基である）の保護されているヌクレオチドである、請求項１に記載の抗体

【請求項６】

前記オリゴヌクレオチドが３～２０のヌクレオチドからなり、前記ヌクレオチドの１つが、式（Ｉ）、

【化５】



(I)

（式中、Ｒは隣接ヌクレオチドとの共有結合であり、
Ｒ１は隣接ヌクレオチドとの共有結合であり、
Ｒ２は－Ｈまたは－ＯＨであり、

塩基はプリンまたはピリミジン塩基であり、
R_4は前記塩基のアミノ基に結合した保護基である）の保護されているヌクレオチドである、請求項１に記載の抗体。
【請求項７】
前記オリゴヌクレオチドが３～２０のヌクレオチドからなり、前記ヌクレオチドの１つが感光性保護基で保護されている、請求項１に記載の抗体。
【請求項８】
抗体がポリクローナル抗体である、請求項１に記載の抗体。
【請求項９】
抗体がモノクローナル抗体である、請求項１に記載の抗体。
【請求項１０】
固体担体に固定した請求項１に記載の抗体。
【請求項１１】
請求項９に記載の抗体を発現する細胞。
【請求項１２】
細胞がハイブリドーマである、請求項１１に記載の細胞。
【請求項１３】
細胞が、前記抗体をコードする異種核酸を含有し、発現する、請求項１１に記載の細胞。
【請求項１４】
請求項１に記載の抗体に合成オリゴヌクレオチドを接触させるステップと、
前記抗体と前記オリゴヌクレオチドとの結合の有無を検出するステップであって、結合の存在が前記合成オリゴヌクレオチドの不完全な脱保護を示すステップと
を含む、イムノアッセイによって合成オリゴヌクレオチドの不完全な脱保護を検出する方法。
【請求項１５】
前記イムノアッセイが不均一なイムノアッセイである、請求項１４に記載の方法。
【請求項１６】
前記イムノアッセイが均一なイムノアッセイである、請求項１４に記載の方法。
【請求項１７】
前記イムノアッセイがサンドイッチアッセイである、請求項１４に記載の方法。
【請求項１８】
前記オリゴヌクレオチドが固体担体に固定されている、請求項１４に記載の方法。
【請求項１９】
完全に脱保護された合成オリゴヌクレオチドから保護されている合成オリゴヌクレオチドを分離する方法であって、
保護されている合成オリゴヌクレオチドと完全に脱保護された合成オリゴヌクレオチドの混合物を請求項１に記載の抗体に接触させるステップであって、前記保護されている合成オリゴヌクレオチドに有機保護基が共有結合されており、その結果前記保護されている合成オリゴヌクレオチドは前記抗体に結合するステップと、
前記完全に脱保護された合成オリゴヌクレオチドから前記抗体を分離するステップと
を含む方法。
【請求項２０】
前記抗体が固体担体に固定されている、請求項１９に記載の方法。
【請求項２１】
前記保護されている合成オリゴヌクレオチドが、部分的に保護されている合成オリゴヌクレオチドである、請求項１９に記載の方法。
【請求項２２】
前記接触させるステップおよび分離するステップがアフィニティークロマトグラフィーによって実施される、請求項１９に記載の方法。
【請求項２３】

イムノアッセイにおいて合成オリゴヌクレオチドの不完全な脱保護を測定するのに有用な物品であって、
少なくとも２つの離れた別個の領域が形成されている表面部分を有する固体担体と、
前記離れた別個の領域の１つに結合した、保護基が結合している第１のオリゴヌクレオチドと、
前記離れた別個の領域の残りの１つに結合した、前記保護基が結合していない第２のオリゴヌクレオチドと
を含み、前記第１および第２のオリゴヌクレオチドのヌクレオチド配列が同じである物品。
【請求項２４】
前記離れた別個の領域の残りの１つに結合した、前記第１のオリゴヌクレオチドに結合している前記保護基が結合している第３のオリゴヌクレオチドをさらに含み、
前記第３のオリゴヌクレオチドが部分的に脱保護されており、
前記第１、第２および第３のオリゴヌクレオチドのヌクレオチド配列が同じである、請求項２３に記載の物品。
【請求項２５】
前記基質がニトロセルロースストリップを含む、請求項２３に記載の物品。
【請求項２６】
有機保護基が共有結合している合成オリゴヌクレオチドに特異的に結合し、前記有機保護基が共有結合していない場合には、抗体が前記合成オリゴヌクレオチドに結合しない抗体を作製する方法であって、
前記有機保護基が共有結合した前記合成オリゴヌクレオチドを固体粒状担体で合成するステップ、または前記有機保護基が結合したヌクレオチドを前記固体担体で合成するステップと、
前記固体担体から前記オリゴヌクレオチドまたはヌクレオチドをはずすことなく、前記抗体を形成するのに十分な量の、前記固体担体に結合した前記合成オリゴマーまたはヌクレオチドにより動物を免疫するステップと
を含む方法。
【請求項２７】
前記合成するステップに続いて、前記免疫するステップの前に、前記ビーズを断片化するステップを実施する、請求項２６に記載の方法。
【請求項２８】
前記動物から前記抗体を回収するステップをさらに含む、請求項２６に記載の方法。
【請求項２９】
前記動物から脾臓細胞を回収するステップと、次いで、
前記脾臓細胞から複数のハイブリドーマ細胞系統を作製するステップと、次いで、
前記複数のハイブリドーマ細胞系統から、前記抗体を作製する特定のハイブリドーマ細胞系統を単離するステップと
をさらに含む請求項２６に記載の方法。
【請求項３０】
前記合成オリゴヌクレオチドが前記固体担体に共有結合している、請求項２６に記載の方法。
【請求項３１】
前記合成オリゴヌクレオチドが、スクシニルリンカーにより前記固体担体に共有結合している、請求項２６に記載の方法。
【請求項３２】
前記固体担体が、コントロールされた孔のガラスビーズを含む、請求項２６に記載の方法。
【請求項３３】
オリゴヌクレオチドの不十分な脱保護または不十分な伸長についてオリゴヌクレオチドア

レイをスクリーニングする方法であって、
（ａ）複数の異なるオリゴヌクレオチドが固定された基板を含むオリゴヌクレオチドアレイを提供するステップであって、前記異なるオリゴヌクレオチドは、前記基板の異なる離れた別個の位置に固定されているステップと、
（ｂ）有機保護基が共有結合した合成オリゴヌクレオチドに特異的に結合し、前記有機保護基が共有結合していない場合には、前記合成オリゴヌクレオチドに結合しない抗体を提供するステップと、
（ｃ）前記オリゴヌクレオチドアレイに前記抗体を接触させ、それによって前記アレイの選択された別個の位置への前記抗体の結合の有無を検出するステップであって、前記アレイの離れた別個の位置への結合の存在がオリゴヌクレオチドの不十分な脱保護または不十分な伸長を示すステップと
を含む方法。
【請求項３４】
前記基板がケイ素を含有する、請求項３３に記載の方法。
【請求項３５】
アレイを提供する前記ステップが、前記基板上で前記オリゴヌクレオチドをｉｎ ｓｉｔｕで合成することによって実施される、請求項３３に記載の方法。
【請求項３６】
アレイのオリゴヌクレオチドにおける複数の異なる保護基を検出できるように、各反復時に異なる抗体を用いてステップ（ｂ）～ステップ（ｃ）を少なくとも１回反復するステップをさらに含む、請求項３３に記載の方法。
【請求項３７】
前記アレイの少なくとも１つの離れた別個の位置のオリゴヌクレオチドの不十分な脱保護または不十分な伸長の存在を記録する証拠を作成するステップをさらに含む、請求項３３に記載の方法。
【請求項３８】
前記証拠が定性的な証拠である、請求項３７に記載の方法。
【請求項３９】
前記証拠が定量的な証拠である、請求項３７に記載の方法。
【請求項４０】
（ａ）複数の異なるオリゴヌクレオチドが固定された基板であって、前記異なるオリゴヌクレオチドが前記基板の異なる離れた別個の位置に固定されている基板と、
（ｂ）前記アレイに関連する複数の証拠であって、前記証拠は少なくとも１つのオリゴヌクレオチドの不十分な脱保護または不十分な伸長の存在を記録し、前記少なくとも１つのオリゴヌクレオチドの各々は、前記アレイの異なる離れた別個の位置に位置していることを特徴とする証拠と
を組み合わせて含む修正可能なオリゴヌクレオチドアレイ。
【請求項４１】
前記基板は、前記基板の異なる離れた別個の位置に少なくとも１０００の異なるオリゴヌクレオチドが固定されている、請求項４０に記載のアレイ。
【請求項４２】
前記証拠が前記アレイに記憶されているか、または印刷されている、請求項４０に記載のアレイ。
【請求項４３】
前記証拠がコンピュータファイルに収納され、前記アレイが、前記基板および前記証拠に関連する識別子をさらに含む、請求項４０に記載のアレイ。
【請求項４４】
前記証拠がウェブサイトに収容され、前記アレイが、前記基板および前記証拠に関連する識別子をさらに含む、請求項４０に記載のアレイ。
【請求項４５】

オリゴヌクレオチドアレイを使用して、前記アレイのオリゴヌクレオチドの不十分な脱保護または不十分な伸長を補正する方法であって、（ａ）複数の異なるオリゴヌクレオチドが固定された基板を提供するステップであって、前記異なるオリゴヌクレオチドが前記基板の異なる離れた別個の位置に固定されているステップと、
（ｂ）前記アレイに関連する証拠を提供するステップであって、前記証拠が少なくとも１つのオリゴヌクレオチドの不十分な脱保護または不十分な伸長の存在を記録し、前記少なくとも１つのオリゴヌクレオチドが前記アレイの離れた別個の位置に位置しているステップと、
（ｃ）試験化合物を提供するステップと、
（ｄ）前記複数の異なるオリゴヌクレオチドの少なくとも１つへの前記試験化合物の結合を検出するステップと、次いで、
（ｅ）（ｉ）前記検出された結合と（ｉｉ）不十分な脱保護または不十分な伸長の存在を記録する前記証拠から、前記オリゴヌクレオチドへの前記試験化合物の結合の程度を測定するステップであって、それによって前記不十分な脱保護または不十分な伸長が前記測定ステップ中に補正されるステップと
を含む方法。
【請求項４６】
前記試験化合物が、タンパク質、ペプチドまたはオリゴヌクレオチドである、請求項４５に記載の方法。
【請求項４７】
前記試験化合物が、ｍＲＮＡである、請求項４５に記載の方法。
【請求項４８】
前記測定するステップが、結合程度の呈色を形成することによって実施される、請求項４５に記載の方法。
【請求項４９】
前記測定するステップが、結合の程度の数値表示を作成することによって実施される、請求項４５に記載の方法。
【請求項５０】
結合の前記程度が、結合親和性、結合量、または結合親和性と結合量の両方である、請求項４５に記載の方法。
【請求項５１】
オリゴヌクレオチドアレイを使用すると同時に前記アレイのオリゴヌクレオチドの不十分な脱保護または不十分な伸長を補正する方法であって、
（ａ）複数の異なるオリゴヌクレオチドが固定されている基板を提供するステップであって、前記異なるオリゴヌクレオチドが前記基板の異なる離れた別個の位置に固定されているステップと、
（ｂ）前記アレイに関連する証拠を提供するステップであって、前記証拠が少なくとも１つのオリゴヌクレオチドの不十分な脱保護または不十分な伸長の存在を記録し、前記少なくとも１つのオリゴヌクレオチドが前記アレイの離れた別個の位置に位置づけられているステップと、
（ｃ）試験化合物を提供するステップと、
（ｄ）前記試験化合物と前記アレイを接触させるステップと、
（ｅ）不十分な脱保護を有する離れた別個の位置における前記少なくとも１つのオリゴヌクレオチドを分析から削除するステップであって、前記アレイへの前記試験化合物の結合が、分析から削除されていない離れた別個の位置上で残存するオリゴヌクレオチドにより検出されるステップと、
（ｆ）前記アレイにおいて離れた別個の位置上で前記残存するオリゴヌクレオチドへの前記試験化合物の結合を検出するステップと
を含む方法。
【請求項５２】

試験化合物が、タンパク質、ペプチドまたはオリゴヌクレオチドである、請求項５１に記載の方法。
【請求項５３】
前記試験化合物が、ｍＲＮＡである、請求項５１に記載の方法。
【請求項５４】
前記検出するステップが、結合の呈色を形成することによって実施される、請求項５１に記載の方法。
【請求項５５】
前記検出するステップが、結合の数値表示を作成することによって実施される、請求項５１に記載の方法。
【発明の詳細な説明】
【０００１】
［関連出願］
本願は、その開示内容が全体として本明細書に参照として組み入れられている、１９９９年１２月３１日に提出された同じ出願人による同時係属出願第０９／４７６，９７５号の一部継続出願である。
【０００２】
［技術分野］
本発明は、オリゴマー、特にオリゴヌクレオチドの化学合成後に残存する保護基の検出と、同定と、定量とに関する。
【０００３】
［背景技術］
この１０年に、ＤＮＡおよびＲＮＡなどの核酸の固体担体での自動化学合成が開発された。これらの化学的方法には、ヌクレオチド塩基アデニン、チミン、シトシンおよびグアニンの環外アミンを保護し、ＲＮＡリボースの２’ＯＨをブロックすることによって合成させる薬品の使用が含まれる。合成の核酸生成物内の塩基は、固体担体から核酸を切断すると脱保護される。しかし、塩基の脱保護程度は容易に測定されない。
【０００４】
例えば、合成ＲＮＡの塩基脱保護後にも、生成物は、リボース部分の２’ＯＨの保護として２’－ジメチルシリルｔｅｒｔ－ブチル基を含有する。この保護基は、ＲＮＡの化学および構造に影響しないように、化学的手段によって慎重に除去される。しかし、２’ＯＨの脱保護の程度は容易に測定されない。核酸は高速液体クロマトグラフィーまたはゲル電気泳動によって精製される。しかし、合成の望ましくない生成物の一部は、１つ以上の保護基および特に５０ヌクレオチドより長いオリゴマーでは、全長の配列からの分離が困難な全長より短い（中断された）配列を含有する完全な核酸配列である。現在、各保護基が、存在する場合には、どの程度生成物に残存するか、生成物のどのくらいの割合が全長であるかを測定する容易な方法はない。一般に、Ｄａｖｉｓ，Ｇ．Ｅ．，Ｇｅｈｒｋｅ，Ｃ．Ｗ．，Ｋｕｏ，Ｋ．Ｃ．，ａｎｄ Ａｇｒｉｓ，Ｐ．Ｆ．（１９７９）Ｍａｊｏｒ ａｎｄ Ｍｏｄｉｆｉｅｄ Ｎｕｃｌｅｏｓｉｄｅｓ ｉｎ ｔＲＮＡ Ｈｙｄｒｏｌｙｓａｔｅｓ ｂｙ Ｈｉｇｈ Ｐｅｒｆｏｒｍａｎｃｅ Ｌｉｑｕｉｄ Ｃｈｒｏｍａｔｏｇｒａｐｈｙ．Ｊ．Ｃｈｒｏｍａｔｏｇｒ．１７３：２８１－２９８；Ａｇｒｉｓ，Ｐ．Ｆ．，Ｔｏｍｐｓｏｎ，Ｊ．Ｇ．，Ｇｅｈｒｋｅ，Ｃ．Ｗ．，Ｋｕｏ，Ｋ．Ｃ．，ａｎｄ Ｒｉｃｅ，Ｒ．Ｈ．（１９８０）Ｈｉｇｈ－Ｐｅｒｆｏｒｍａｎｃｅ Ｌｉｑｕｉｄ Ｃｈｒｏｍａｔｏｇｒａｐｈｙ ａｎｄ Ｍａｓｓ Ｓｐｅｃｔｒｏｍｅｔｒｙ ｏｆ Ｔｒａｎｓｆｅｒ ＲＮＡ Ｂａｓｅｓ ｆｏｒ Ｉｓｏｔｒｏｐｉｃ Ａｂｕｎｄａｎｃｅ．Ｊ．Ｃｈｒｏｍａｔｏｇｒ．１９４：２０５－２１２；Ｇｅｈｒｋｅ，Ｃ．Ｗ．，Ｋｕｏ，Ｋ．Ｃ．，ＭｃＣｕｎｅ，Ｒ．Ａ．，Ｇｅｒｈａｒｄｔ，Ｋ．Ｏ．，ａｎｄ Ａｇｒｉｓ，Ｐ．Ｆ．（１９８１）Ｑｕａｎｔｉｔａｔｉｖｅ Ｅｎｃｚｙｍａｔｉｃ Ｈｙｄｒｏｌｙｓｉｓ ｏｆ ｔＲＮＡｓ：ＲＰ－ＨＰＬＣ ｏｆ ｔＲＮＡ Ｎｕｃｌｅｏｓｉｄｅｓ．Ｊ．Ｃｈｒｏｍａｔｏｇｒ．２３０：２９７－３０８；Ｃｈｒｏｍａｔ

ography and Modification of Nucleosides Volumes A, B and C（Gehrke, C. W. and Kuo, K. C. T., eds）, Elsevier Publishing Co. 1990；Agris, P. F. and Sierzputowska－Gracz, H.（1990）Three Dimensional Dynamic Structure of tRNA's by Nuclear Magnetic Resonance. In Chromatography and Modification of Nucleosides（Gehrke, C. W. and Kuo, K. C. T., eds.）, Elsevier Publishing Co., pp. 225－253；Agris, P. F., Hayden, J., Sierzputowska－Gracz, H., Ditson, S., Degres, J. A., Tempesta, M., Kuo, K. C. and Gehrke and Gehrke, C. W.（1990）Compendium on Biological, Biochemical, Chemical, Physical and Spectroscopic Properties of RNA and DNA Nucleosides. In Chromatography and Modification of Nucleosides, Elsevier Publishing Co. 参照。

【０００５】

保護基の除去が不完全であることおよび簡単なアッセイがないことは、２つの業界にとって、また全世界の数多くの研究者にとって問題である。（ｉ）現在翌日渡しにより核酸配列合成を提供している多数の会社は、生成物が脱保護された程度を顧客に伝えることが難しい、（ｉｉ）製薬会社は、導入または販売しようとしている治療用または診断用オリゴヌクレオチド生成物の純度および／または鎖長を規制機関に対して容易には証明できない。従って、全長のオリゴヌクレオチド生成物の純度および割合を測定する簡単で信頼できる方法の必要性がある。

【０００６】

［発明の開示］

本発明の一態様は、有機保護基が共有結合している合成オリゴマー（すなわち、オリゴヌクレオチドまたはオリゴペプチド）に特異的に結合し、有機保護基が共有結合していない場合にはその合成オリゴマーに結合しない抗体（例えば、モノクローナル抗体またはポリクローナル抗体）である。

【０００７】

本発明の第２の態様は、上記の抗体を発現する、細胞培養物および単離細胞を含む細胞を含む。このような細胞は、抗体を発現する異種核酸を含有し、発現するハイブリドーマ細胞および組み換え細胞を含む。

【０００８】

本発明の第３の態様は、（ａ）上記の抗体に合成オリゴヌクレオチドを接触させるステップと、（ｂ）前記抗体と前記オリゴマーとの結合の有無を検出するステップであって、結合の存在が前記合成オリゴマーの不完全な脱保護を示すステップとを含む、イムノアッセイによって合成オリゴヌクレオチドの不完全な脱保護を検出する方法である。不均一な（ｈｅｔｅｒｏｇｅｎｅｏｕｓ）イムノアッセイおよび均一な（ｈｏｍｏｇｅｎｅｏｕｓ）イムノアッセイを含む、任意の好適なアッセイ方式を使用することができる。例えば、イムノアッセイはイムノブロットードットアッセイであっても、サンドイッチアッセイであってもよい。

【０００９】

本発明の第４の態様は、完全に脱保護された合成オリゴマーから（部分的および完全に保護されている合成オリゴマーを含む）保護されている合成オリゴマーを分離する方法である。本発明の方法は、（ａ）保護されている合成オリゴマーと完全に脱保護された合成オリゴマーの混合物を上記の抗体に接触させるステップであって、保護されている合成オリゴマーは有機保護基が共有結合されており、その結果、保護されている合成オリゴマーが

抗体に結合するステップと、完全に脱保護されたオリゴマーから抗体を分離するステップとを含む。抗体は、分離を容易にするために、固体担体に固定されてもよい。保護されている合成オリゴマーは、部分的に保護されている合成オリゴマー（用途の１つは、全長および中断された（ａｂｏｒｔｅｄ）配列オリゴマーの同定および／または精製である）または脱保護を受けていない完全に保護されている合成オリゴマーであってもよい。アフィニティークロマトグラフィーを含むが、これに限定されない任意の分離方式を使用することができる。

【００１０】

本発明の第５の態様は、イムノアッセイにおいて合成オリゴマーの不完全な脱保護を測定するのに有用な物品であって、（ａ）少なくとも２つの離れた別個の領域が形成された表面部分を有する固体担体（例えば、ニトロセルロースストリップ）と、（ｂ）前記離れた別個の領域の１つに結合し、保護基が結合されている第１のオリゴマーと、（ｃ）前記離れた別個の領域の別の１つに結合し、前記保護基が結合されていない第２のオリゴマーとを含み、前記第１および第２のオリゴマーのヌクレオチド配列が同じである物品である。好ましい実施態様において、物品は、（ｄ）前記離れた別個の領域の別の１つに結合し、前記第１のオリゴマーに結合している前記保護基が結合している第３のオリゴマーをさらに含み、前記第３のオリゴマーが部分的に脱保護されており、前記第１、第２および第３のオリゴマーのヌクレオチド配列が同じである。

【００１１】

本発明の第６の態様は、有機保護基が共有結合した合成オリゴマーに特異的に結合し、前記有機保護基が共有結合していない場合には前記合成オリゴマーに結合しない抗体を作製する方法であって、（ａ）前記有機保護基が共有結合した（好ましくは、スクシニルリンカーで共有結合した）前記合成オリゴマーを固体粒状担体で合成するステップ（または、前記保護基が共有結合した１ヌクレオチドのモノマーを固体担体で合成するステップ）と、前記オリゴマーを前記固体担体からはずすことなく、（ｂ）前記抗体を形成するのに十分な量の、前記固体担体に結合した前記合成オリゴマー（または、前記固体担体に結合したモノマー）で動物を免疫するステップを含む方法である。必要に応じて、固体担体は、タンパク質（例えば、ウシ血清アルブミン）などの担体グループと交換してもよい。

【００１２】

要約すると、本発明の抗体および方法は、有機合成方法に使用される保護基の定性および定量的検出などのイムノアッセイに有用であり、研究、治療、診断および生物医科学にオリゴヌクレオチドまたはペプチドの特定の用途がある。本発明の抗体は、副産物夾雑物からの最終生成物の分離などの精製技法に使用することができる。本発明は、遺伝子治療の薬剤、アンチセンス、ａｎｔｉｇｅｎｅの品質管理および遺伝子発現のコントロール、保護基を含有してもよい生物医学ポリマーの品質管理、並びに合成オリゴマー、特にオリゴヌクレオチドまたはペプチドの精製および特徴づけなどの、オリゴヌクレオチドおよびペプチド合成の品質管理過程に使用することができる。

【００１３】

本発明は、本明細書の図面および以下に記載する明細書により詳細に説明されている。

【００１４】

［好ましい実施態様の詳細な説明］

１．一般的な定義

本明細書において使用される「抗体」は、モノクローナル抗体およびポリクローナル抗体の両方をいい、（ＩｇＧおよびＩｇＭ抗体を含むが、これらに限定されない）任意の免疫グロブリン型の抗体をいい、高頻度可変領域または結合領域を保持する抗体断片を含む。抗体はいかなる起源のものであってもよいが、典型的には、哺乳類である（例えば、ウマ、ラット、マウス、ウサギ、ヤギ）。抗体は、既知の技法により、ニトロセルロース、アガロース、ガラス、有機ポリマー（「プラスチック」）等に結合または固定されてもよく、既知の技法により、他の検出可能な基で標識されても、または検出可能な基に接続されてもよい。

【００１５】
抗体のオリゴマーへの選択的な結合に関して本明細書において使用される「結合」は当技術上通常の意味を有する。一般に、イムノアッセイまたは親和性精製技法において識別するために、抗体は、少なくとも約K_d＝10^{-6}、10^{-7}または10^{-8}Mの親和性で保護されているオリゴマーに結合するべきであり、約K_d＝10^{-2}、10^{-3}または10^{-4}Mを超えない親和性で保護されていないオリゴマーと結合するべきである。
【００１６】
本明細書において使用される「オリゴマー」は、ＤＮＡおよびＲＮＡなどの天然型の形態の合成オリゴマー、並びに以下に考察する修飾された骨格の化合物を含む、合成オリゴヌクレオチドおよび合成オリゴペプチドをいう。オリゴヌクレオチドは本発明を実施する際に現在好ましく、本発明は、本明細書のオリゴヌクレオチドに言及して主に説明されている。しかし、本明細書に記載されている方法および技法はまたオリゴペプチド、オリゴサッカライド等（すなわち、合成に保護基を必要とする、合成によって生成される任意のポリマー）に適用することもできる。
【００１７】
本明細書において使用される「ヌクレオチド」は、ペントース、含窒素複素環塩基（典型的には、ペントースの１位に結合している）およびリン酸塩またはリン酸基（典型的には、ペントースの５’位に結合している）を含むオリゴヌクレオチドのサブユニットをいうが、オリゴヌクレオチドの５’末端ヌクレオチドの３’位が欠損しているかまたは３’位に結合していると考えられる。これらの構造は周知である。例えば、Ａ．Ｌｅｈｎｉｎｇｅｒ，Ｂｉｏｃｈｅｍｉｓｔｒｙ，３０９－３２０を参照）。「ヌクレオシド」は、典型的には、リン酸またはリン酸塩を欠損しているヌクレオチドをいう。
【００１８】
本明細書において使用される「保護基」は当技術上従来の意味を持ち、（典型的には、有機合成において）その分子が関係する化学反応の前に、分子中の原子に結合、典型的には、共有結合し、その結果、保護基が結合している原子に化学反応が起きない化学的部分、基または置換基をいう。典型的には、最終生成物が生成されるために、保護基は中間体分子から化学的に除去されるが、除去技法により最終生成物の部分的な脱保護（すなわち、その分子に残存する少なくとも１つの保護基が存在する）だけが生じるわけではないことがある。保護基は、上記の抗体を作製または試験する目的のために分子に意図的に残されることがある。
【００１９】
本明細書において使用される「脱保護」または「脱保護された」は、分子からの化学的なオリゴヌクレオチド合成に使用される保護基がないことをいう。このような保護基は以下に記載されている。保護基が鎖の末端である場合には、このような保護基の存在はオリゴヌクレオチドの不十分な伸長を示すことができる。化学的に合成されたオリゴヌクレオチドは、理想的には、完全に脱保護されているが、本発明は、このようなオリゴヌクレオチドの部分的または不完全な脱保護（すなわち、オリゴヌクレオチドに以下に記載する少なくとも１つの保護基が存在する）を検出するために使用される。
【００２０】
オリゴヌクレオチドに関して本明細書において使用される「塩基」は、プリン（例えば、アデニン、グアニン）またはピリミジン（例えば、ウラシル、チミン、シトシン）の誘導体であるまたは含窒素複素環塩基をいう。ピリミジン塩基は、１環窒素によってペントースに結合し、プリン塩基は９環窒素によってペントースに結合する。好ましい塩基は、グアニン、アデニンおよびシトシンなどの遊離アミノ基を含有するものである（次いで、遊離アミノ基の水素の１つまたは２つの置換によって、保護基が遊離アミノ酸基に共有結合される）。しかし、保護するための遊離アミノ基または合成中に保護を必要とする他の基をオリゴヌクレオチド中に含有する、標準的または修飾／稀（ｒａｒｅ）な任意のプリンまたはピリミジン塩基に使用することができる。標準的および修飾／稀な塩基の例は以下の表１に記載されているヌクレオシドに見られるものである。

【００２１】
【表１】

表１　標準的および修飾ヌクレオシド並びに標準的な略語

略語	塩基
U	ウリジン
C	シチジン
A	アデノシン
G	グアノシン
T	チミジン
?A	未知の修飾アデノシン
m1A	１－メチルアデノシン
m2A	２－メチルアデノシン
i6A	N^6－イソペンテニルアデノシン
ms2i6A	２－メチルチオ－N^6－イソペンテニルアデノシン
m6A	N^6－メチルアデノシン
t6A	N^6－スレオニルカルバモイルアデノシン
m6t6A	N^6－メチル－N^6－スレオニルカルバモイルアデノシン
ms2t6A	２－メチルチオ－N^6－スレオニルカルバモイルアデノシン
Am	２’－Ｏ－メチルアデノシン
I	イノシン
m1I	１－メチルイノシン
Ar(p)	２’－Ｏ－（５－ホスホ）リボシルアデノシン
io6A	N^6－（ｃｉｓ－ヒドロキシイソペンテニル）アデノシン
?C	未知の修飾シチジン
s2C	２－チオシチジン
Cm	２’－Ｏ－メチルシチジン
ac4C	N^4－アセチルシチジン
m5C	５－メチルシチジン
m3C	３－メチルシチジン
k2C	リシジン
f5C	５－ホルミルシチジン
f5Cm	２’－Ｏ－メチル－５－ホルミルシチジン
?G	未知の修飾グアノシン
Gr(p)	２’－Ｏ－（５－ホスホ）リボシルグアノシン
m1G	１－メチルグアノシン
m2G	N^2－メチルグアノシン
Gm	２’－Ｏ－メチルグアノシン
m22G	N^2N^2－ジメチルグアノシン
m22Gm	N^2，N^2，２’－Ｏ－トリメチルグアノシン
m7G	７－メチルグアノシン
fa7d7G	アルカエオシン（archaeosine）
Q	クエノシン（quenosine）
manQ	マンノシル－クエノシン（quenosine）
galQ	ガラクトシル－クエノシン（quenosine）
Yw	ワイブトシン
o2yW	ペルオキシワイブトシン
?U	未知の修飾ウリジン

【表１（つづき）】

略語	塩基
ｍｎｍ５Ｕ	５－メチルアミノメチルウリジン
ｓ２Ｕ	２－チオウリジン
Ｕｍ	２’－Ｏ－メチルウリジン
ｓ４Ｕ	４－チオウリジン
ｎｃｍ５Ｕ	５－カルバモイルメチルウリジン
ｍｃｍ５Ｕ	５－メトキシカルボニルメチルウリジン
ｍｎｍ５ｓ２Ｕ	５－メチルアミノメチル－２－チオウリジン
ｍｃｍ５ｓ２Ｕ	５－メトキシカルボニルメチル－２－チオウリジン
ｃｍｏ５Ｕ	ウリジン５－オキシ酢酸
ｍｏ５Ｕ	５－メトキシウリジン
ｃｍｎｍ５Ｕ	５－カルボキシメチルアミノメチルウリジン
ｃｍｎｍ５ｓ２Ｕ	５－カルボキシメチルアミノメチル－２－チオウリジン
ａｃｐ３Ｕ	３－（３－アミノ－３－カルボキシプロピル）ウリジン
ｍｃｈｍ５Ｕ	５－（カルボキシヒドロキシメチル）ウリジンメチルエステル
ｃｍｎｍ５Ｕｍ	５－カルボキシメチルアミノメチル－２’－Ｏ－メチルウリジン
ｎｃｍ５Ｕｍ	５－カルバモイルメチル－２’－Ｏ－メチルウリジン
Ｄ	ジヒドロウリジン
Ψ	シュードウリジン
ｍ１Ψ	１－メチルシュードウリジン
Ψｍ	２’－Ｏ－メチルシュードウリジン
ｍ５Ｕ	リボシルチミン
ｍ５ｓ２Ｕ	５－メチル－２－チオウリジン
ｍ５Ｕｍ	５，２’－Ｏ－ジメチルウリジン

Ｓｐｒｉｎｚｌ ｅｔ ａｌ．，Ｎｕｃｌｅｉｃ Ａｃｉｄｓ Ｒｅｓ．２６，１４８（１９９８）参照。

【００２２】
出願人らは、本明細書に引用されている全ての米国特許参照文献の開示内容が全体として本明細書に参照として組み入れられていることを意図している。

【００２３】
２．保護基
特定の保護基は、合成されるオリゴマーおよびそのオリゴマーが合成される方法に依存する。

【００２４】
オリゴヌクレオチドの合成に好適な保護基には、Ｎ、ＯまたはＳなどの１つ以上のヘテロ原子を含有してもよく、置換または未置換であってもよい（例えば、カルボニル基）アルキル、アリール（ａｒｙｌ）、アルキルアリール、アリールアルキル基が含まれる。保護基の例には、以下を含むが、これらに限定されない：アセチル、イソブチリル、２－（ｔ－ブチルジフェニル－シリルオキシメチル）ベンゾイル、ナフタロイル、イソ－ブチリルオキシカルボニル、レブリニル（ｌｅｖｕｌｉｎｙｌ）、フルオレニルメトキシカルボニル、２－ニトロチオフェニル、２，２，２－トリクロロ－ｔ－ブトキシカルボニル、エトキシカルボニル、ベンジルオキシカルボニル、ｐ－ニトロフェニル－エチルオキシカルボニル、Ｎ’Ｎ－ジメチルホルムアミジン、ホルミル、ベンゾイル、トルイル、２，４－６－トリメチルベンゾイル、アニソイル、２，４－ジメチルフェニル、２，４，６－トリメチルフェニル、トリフェニルチオメチル、ピボロイルオキシメチル、ｔ－ブトキシカルボニル、ｐ－ニトロフェニルエチル、メトキシエトキシメチル、ブチルチオカルボニル、２－メチル－ピリジン－５－イル、２－ニトロチオフェニル、２，４－ジニトロチオフェニル、２－ニトロ－４－メチルチオフェニル、ｐ－ニトロフェニルスルホニルエチル、５－クロロ－８－ヒドロキシキノリン、チオフェニル、β－シアノエチル、フェニルエチル、

ｐ－ニトロフェニルエチル、ピリジルエチル、２－Ｎ－メチルイミダゾリルフェニル、メチル、アリル（ａｌｌｙｌ）、トリクロロエチル、ジベンゾイル、ｐ－ニトロフェニルエトキシカルボニル、ベンゾイルおよびその置換誘導体、２（アセトキシメチル）ベンゾイル、４，４’、４’’－トリス（ベンジルオキシ）トリチル、５－メチルピリジノ（ｐｙｒｉｄｙｎｏ）－２－イル、フェニルチオエチル、ジフェニルカルバモイル、３，４－ジメトキシベンジル、３－クロロフェニル、２－ニトロフェニル、９－フェニルキサンテン－９－イル、９－（ｐ－メトキシフェニル）キサンテン－９－イル、９－（ｐ－オクタデシルオキシフェニル）キサンテン－９－イル、「架橋」ビス－ジメトキシトリチル基、フタノイル、スクシニル、ベンゼンスルホニルエトキシカルボニル、４，４’、４’’－トリス（ベブリニルオキシ（ｂｅｖｕｌｉｎｙｌｏｘｙ））トリストリチル、ｐ－フェニルアゾフェニルオキシカルボニル、ｏ－置換ベンゾイル、４，４’、４’’－トリス（４，５－ジクロロファルイミジン（ｐｈａｌｉｍｉｄｉｎ）トリチル、レベリニル（ｌｅｖｅｌｉｎｙｌ）、アルキルオキシおよびアリールオキシアセチル、１，３－ベンゾジチオール－２－イル、テトラヒドロフラニル、［２－（メチルチオ）フェニル］チオメチル、１－（２－クロロエチ（ｅｔｈｙ）オキシ）エチル、１－［（２－フルオロ－フェニル］４－メトキシピペリジン－４－イル、４－メトキシテトラヒドロピラン－４－イル、（１－メチル－１－メトキシ）エチル、テトラヒドロピラニル、３－メトキシ－１，５－ジカルボメトキシペンタム（ｐｅｎｔａｍ）－３－イル、２－ニトロベンジル、ベンジル、４－ニトロフェニルエチル－スルホニル、ｔ－ブチルジメチルシリル、４－メトキシベンジル、３，４－ジメトキシベンジル、９－ｐ－メトキシフェニルチオキサンテン－９－イル、式$R_1R_2R_3C$－（ここで、R_1、R_2およびR_3は、各々独立に、フェニル、ｐ－モノメトキシフェニル、ｏ－モノメトキシフェニル、ビフェニル、ｐ－フルオロフェニル、ｐ－クロロフェニル、ｐ－メチルフェニル、ｐ－ニトロフェニル等からなる群から選択される）の化合物。

【００２５】

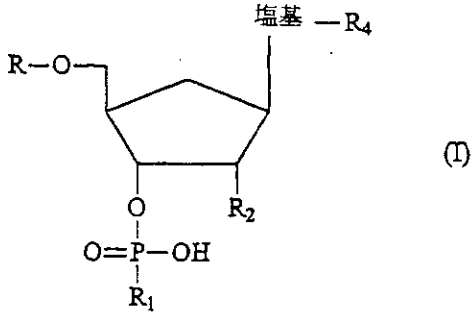
３．オリゴヌクレオチド

保護基を含有し、本発明を実施するために使用することができる合成オリゴヌクレオチドには、ＤＮＡおよびＲＮＡなどの天然型、並びにホスホネート、ホスホールアミド、ホスホンアミド、ホスファイト、ホスフィンアミド等、などのポリ（ホスフェート誘導体）スルホン、スルホネート、スルファイト、スルホンアミド、スルフェンアミド（ｓｕｌｆｅｎａｍｉｄｅｓ）のようなポリ（硫黄誘導体）等などの修飾された骨格の化合物が含まれる。本発明の抗体は特定の「試薬」または「ベンチマーク」オリゴヌクレオチドとの選択的な結合によって特徴づけることができるが、同じ抗体は、同じ保護基を含有する種々の他のオリゴヌクレオチド（例えば、より長いヌクレオチド）または他の化合物にも結合することができるということが注目される。

【００２６】

例えば、抗体が選択的に結合するオリゴヌクレオチドは、３～２０のヌクレオチドからなってもよく、前記ヌクレオチドの１つは以下の式（Ｉ）、

【化６】



(I)

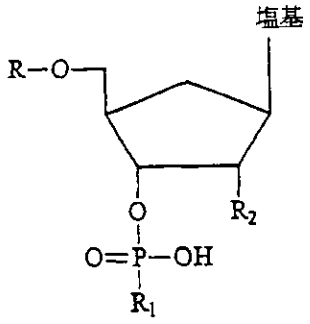
（式中、ＲはＨまたはジメトキシトリチルなどの保護基であるが、ただし、前記保護されている塩基が前記オリゴヌクレオチドの５’末端ヌクレオチドでない場合には、Ｒは隣接

ヌクレオチドとの共有結合であり、
R_1はＨまたはβ－シアノエチルなどの保護基であるが、ただし、前記保護されている塩基が前記オリゴヌクレオチドの３’末端ヌクレオチドでない場合には、R_1は隣接ヌクレオチドとの共有結合であり、
R_2はＨまたは－OR_3であり、
R_3はＨまたはｔｅｒｔ－ブチルジメチルシリルなどの保護基であり、
塩基はプリンまたはピリミジン塩基であり、
R_4は、アセチル（Ａｃ）、ベンゾイル（Ｂｚ）、ジメチルホルムアミジン（ｄｍｆ）、イソブチル（ｉｓｏｂｕｔｙｒｌ）（Ｉｂｕ）、フェノキシアセチル（Ｐａｃ）およびイソプロピル－フェノキシアセチル（ｉｐｒ－Ｐａｃ）からなる群から選択される保護基などの前記塩基のアミノ基に結合した保護基であるが、ただし、Ｒ、R_1、R_3およびR_4の１つが保護基である場合には、Ｒ、R_1、R_3およびR_4の残りは保護基でない）の保護されたヌクレオチドである。

【００２７】

上記の１つの特定の実施態様において、抗体は、３～２０ヌクレオチドからなり、式（Ｉ）、

【化７】



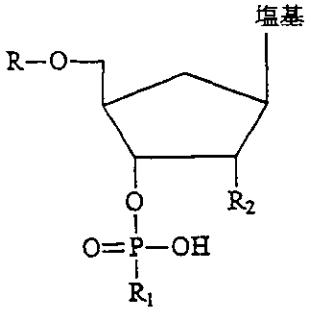
(I)

（式中、Ｒはジメトキシトリチルなどの保護基であり、
R_1は隣接ヌクレオチドとの共有結合であり、
R_2は－Ｈまたは－ＯＨであり、
塩基はプリンまたはピリミジン塩基である）の保護されたヌクレオチドである５’ヌクレオチドを有するオリゴヌクレオチドに選択的に結合するものであってもよい。

【００２８】

上記の別の特定の実施態様において、抗体は、３～２０ヌクレオチドからなり、式（Ｉ）、

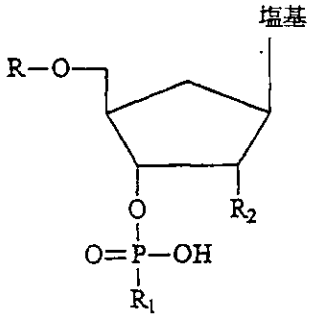
【化８】



(I)

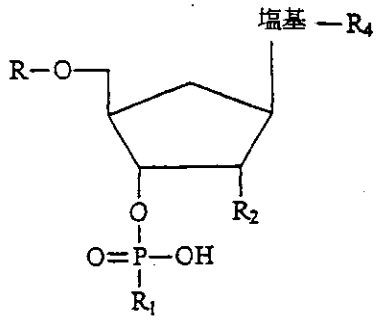
（式中、Ｒは隣接ヌクレオチドとの共有結合であり、
R_1はβ－シアノエチルなどの保護基であり、
R_2は－Ｈまたは－ＯＨであり、
塩基はプリンまたはピリミジン塩基である）の保護されたヌクレオチドである３’ヌクレオチドを有するオリゴヌクレオチドに選択的に結合するものであってもよい。

【００２９】
上記の別の特定の実施態様において、抗体は、３～２０ヌクレオチドからなり、前記ヌクレオチドの１つは、式（Ｉ）、
【化９】



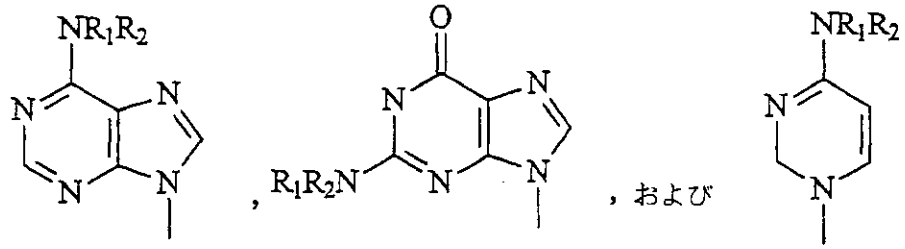
(I)

（式中、Ｒは隣接ヌクレオチドとの共有結合であり、
R_1 は隣接ヌクレオチドとの共有結合であり、
R_2 は－OR_3 であり、
R_3 はｔｅｒｔ－ブチルジメチルシリルなどの保護基であり、
塩基はプリンまたはピリジン塩基である）の保護されたヌクレオチドであるオリゴヌクレオチドに選択的に結合するものであってもよい。
【００３０】
上記のさらに別の特定の実施態様において、抗体は、３～２０ヌクレオチドからなり、前記ヌクレオチドの１つは、式（Ｉ）、
【化１０】



(I)

（式中、Ｒは隣接ヌクレオチドとの共有結合であり、
R_1 は隣接ヌクレオチドとの共有結合であり、
R_2 は－Ｈまたは－ＯＨであり、
塩基はプリンまたはピリジン塩基であり、
R_4 は、アセチル、ベンゾイル、ジメチルホルムアミジン、イソブチリル、フェノキシアセチルおよびイソプロピル－フェノキシアセチルなどの、前記塩基のアミノ基に結合した保護基である）の保護されたヌクレオチドであるオリゴヌクレオチドに選択的に結合するものであってもよい。
【００３１】
従って、上記に示す構造に使用することができる保護された塩基の例には、以下の、
【化１１】



（式中、R_1およびR_2は保護されていない塩基ではHであり、保護されている塩基では、R_1またはR_2は、上記の保護基（例えば、Ｐａｃ、Ｉｐｒ－Ｐａｃ、Ｉｂｕ、Ｂｚ、Ａｃ、ｄｍｆ）である）のようなアデニン、グアニンおよびシトシンが含まれるが、これらに限定されない。同様に、修飾されているヌクレオチドは、化学的に反応性である修飾基に保護基を有する。

【００３２】

本発明の一実施態様において、オリゴヌクレオチドはペプチド核酸であり、保護基は、米国特許第６，１３３，４４４号に記載されているものを含むが、これらに限定されないペプチド核酸の合成に使用されるような保護基である。

【００３３】

上記のさらに別の特定の実施態様において、抗体は３～２０ヌクレオチドからなり、前記ヌクレオチドの１つは、米国特許第５，７４４，１０１号および同第５，４８９，６７８号（Ａｆｆｙｍａｘに付与されている）に記載されているものを含むが、これらに限定されない感光性保護基で保護されているオリゴヌクレオチドに選択的に結合するものであってもよい。

【００３４】

４．抗体

上記のように、本発明は、有機保護基が共有結合した合成オリゴヌクレオチドに特異的に結合し、前記有機保護基が共有結合していない場合には前記合成オリゴヌクレオチドに結合しない抗体（例えば、モノクローナル抗体またはポリクローナル抗体）を提供する。

【００３５】

抗体は、既知の技法により固体担体に固定（または結合）された状態で提供されても、遊離の結合していない形態（例えば、凍結乾燥、凍結、水性担体中等）で提供されてもよい。抗体が固定されるかどうかは、抗体を使用する特定のイムノアッセイまたは親和性精製技法に依存し、このような技法の既知のパラメーターによって決定される。同様に、典型的には、抗体を使用するイムノアッセイ方式に応じて、酵素（例えば、西洋ワサビペルオキシダーゼ）、ビオチンまたはアビジンなどの数多くの結合対、放射性基または緑色蛍光タンパク質などの蛍光基などの好適な検出可能な基を既知の技法により抗体に結合または接合することができる。

【００３６】

５．イムノアッセイ方法

本発明は、イムノアッセイによって（保護基を含有する中断された（ａｂｏｒｔｅｄ）配列を含む）合成オリゴヌクレオチドの不完全な脱保護を検出する方法を提供する。一般に、このようなイムノアッセイは、（ａ）上記の抗体に合成オリゴヌクレオチドを接触させるステップと、（ｂ）前記抗体と前記オリゴヌクレオチドとの結合の有無を検出するステップであって、結合の存在が前記合成オリゴヌクレオチドの不完全な脱保護を示すステップとを含む。不均一なイムノアッセイおよび均一なイムノアッセイを含む、任意の好適なアッセイ方式を使用することができる。例えば、イムノアッセイはイムノドット－ブロットアッセイであっても、またはサンドイッチアッセイであってもよい。脱保護について試験されるオリゴヌクレオチドは、溶液または固体担体に固定された形態などの任意の好適な形態であってもよい。

【００３７】

好ましい実施態様において、検出方法は、抗体と試験オリゴヌクレオチドとの結合を、抗体と既知のオリゴヌクレオチドセットとの結合と比較し、全てが共通の固体担体に固定されている「ディップスティック」等を使用する。イムノアッセイにおいて合成オリゴヌクレオチドの不完全な脱保護を測定するのに有用な、図１０に例示するような物品は、（ａ）少なくとも２つの離れた別個の領域２６および２７が形成されている表面部分を有する固体担体（例えば、ニトロセルロースストリップ）２５と、（ｂ）前記離れた別個の領域の１つに結合し、保護基が（例えば、少なくとも１つの保護基）結合している第１のオリゴヌクレオチドと、（ｃ）前記離れた別個の領域の別の１つに結合し、前記保護基が結合していない第２のオリゴヌクレオチドとを含み、前記第１および第２のオリゴヌクレオチドのヌクレオチド配列が同じである。好ましい実施態様において、本発明の物品は、（ｄ）前記離れた別個の領域２８の別の１つに結合し、前記第１のオリゴヌクレオチドに結合している前記保護基が結合している第３のオリゴヌクレオチドをさらに含み、前記第３のオリゴヌクレオチドが部分的に脱保護されており（すなわち、共有結合されている保護基の数が、第１のオリゴヌクレオチドと第２のオリゴヌクレオチドに結合しているものの中間であり、例えば、保護基が第１のオリゴヌクレオチドより少なくとも１、２、３または４つ以上多く、第１のオリゴヌクレオチドより少なくとも１０、２０以上多い）、前記第１、第２および第３のオリゴヌクレオチドのヌクレオチド配列が同じである。当然のことであるが、望ましい場合には、別の離れた別個の位置の基板に、異なる数の保護基を有するさらに多くのオリゴヌクレオチドを含んでもよい。別のオリゴヌクレオチドが結合する別個の領域は、ドットなどの任意の形態であってもよい。

【００３８】

６．親和性精製方法

イムノアッセイ以外に、本発明はまた、完全に脱保護されたオリゴヌクレオチドを、部分的に脱保護されたオリゴヌクレオチド（完全に保護されているオリゴヌクレオチドを含む）（例えば、保護基を除去するために脱保護過程を実施したオリゴヌクレオチドと脱保護過程を実施していないオリゴヌクレオチドの両方）から分離する親和性精製技法を提供する。このような手法は、典型的には、（ａ）保護されている合成オリゴヌクレオチドと完全に脱保護された合成オリゴヌクレオチドの混合物を上記の抗体に接触させるステップであって、保護されている合成オリゴヌクレオチドは、抗体が選択できる有機保護基が共有結合されており、その結果、保護されている合成オリゴヌクレオチドが抗体に結合するステップと、次いで前記抗体を前記完全に脱保護されたオリゴヌクレオチドから分離するステップとを含む。保護されている合成オリゴヌクレオチドは部分的に保護されている合成オリゴヌクレオチドであっても、脱保護を受けていない完全に保護されている合成オリゴヌクレオチドであってもよい。アフィニティークロマトグラフィーを含むが、これに限定されない任意の分離方式を使用することができる。

【００３９】

７．抗体の作製

有機保護基が共有結合している合成オリゴヌクレオチドに特異的に結合し、前記有機保護基が共有結合していない場合には、前記合成オリゴヌクレオチドに結合しない抗体を作製する方法は、（ａ）有機保護基が共有結合した合成オリゴヌクレオチドを（好ましくは、例えば、スクシニルリンカーで共有結合している）固体粒状担体で合成するステップと、前記固体担体からオリゴヌクレオチドまたはヌクレオチドをはずすことなく、（ｂ）抗体を形成するのに十分な量の、固体担体に結合した合成オリゴヌクレオチドで動物を免疫するステップとを含む。また、有機保護基が結合している１つのヌクレオチドを固体粒状担体に結合してもよく、本明細書に上記するように使用することができる。

【００４０】

合成ステップは、既知の技法により固体担体上で実施することができる。固体担体は合成前は粒状形態であってもよく、合成後は粒子に断片化されてもよい。一般に、固体担体は、全体が完全に固体であっても、多孔性であっても、変形可能であっても、または硬くてもよいビーズである。ビーズは、一般に、直径が少なくとも１０、２０または５０～２５

０、５００または２０００μｍであり、最も典型的には、直径５０～２５０μｍである。セルロース、多孔性ガラス、シリカゲル、ジビニルベンゼンを架橋したポリスチレンビーズなどのポリスチレンビーズ、ポリエチレングリコール／ポリスチレンなどのグラフトコポリマービーズ、ポリアクリルアミドビーズ、ラテックスビーズ、ジメチルアクリルアミドビーズ、直鎖状ポリスチレンを接続した架橋ポリスチレンまたはフッ素化エチレンポリマーなどの疎水性ポリマーをコーティングしたガラスビーズなどの複合体等を含む、任意の便利な組成物を固体担体に使用することができる。粒子またはビーズなどの離れた別個の個体担体を使用する場合には、それらは、一般に、総反応混合物の約１～９９重量パーセントを含む。

【００４１】

好ましい実施態様において、合成ステップの次で、免疫ステップの前に、固体担体を（例えば、粉砕によって）断片化するステップを実施する。ポリクローナル抗体を既知の技法により動物の血清から回収するか、または脾臓細胞を動物から回収してもよく、複数のハイブリドーマ細胞系統を脾臓細胞から作製し、次いで抗体を作製する特定のハイブリドーマ細胞系統を複数のハイブリドーマ細胞系統から単離することができる。

【００４２】

核酸および他の合成に使用される保護基に対する抗血清／ポリクローナル抗体およびモノクローナル抗体を作製する特定のプロトコールは、典型的には、以下のステップを含む。（ａ）保護基を含有する、または含有しないオリゴヌクレオチドおよび他を作製するステップ、（ｂ）そのような調製物で動物を免疫するステップ、（ｃ）保護基に対する抗体を示すものを同定するために動物をスクリーニングするステップ、（ｄ）典型的な融合方法によってモノクローナル抗体を作製するステップと、（ｅ）必要に応じて、抗体操作によってｓｃＦａｂ、Ｆａｂ断片および抗体分子全体を作製するステップ、および（ｆ）保護基に対するモノクローナル抗体を評価し、特徴づけるステップ。これらのステップの各々は以下にさらに詳細に考察されている。

【００４３】

保護基を含有する合成オリゴヌクレオチドは、当業者に既知の種々の方法で合成することができる。例えば、細孔性ガラス（Ｃｏｎｔｒｏｌｌｅｄ　ｐｏｒｅ　ｇｌａｓｓ（ＣＰＧ））ビーズに接続している個々のヌクレオチドに保護基を結合することができる。例として以下が挙げられる：

ＣＰＧビーズ－ｄＴ（ＤＭＴ基のみ）。

【００４４】

別の方法として、ＣＰＧビーズに接続しているオリゴヌクレオチド鎖に保護基を結合することができる。例として以下が挙げられる：

Ｂｚ－ｄＣおよびＩｂｕ－ｄＧによるＰａｃ－ｄＡ－Ｐａｃ－ｄＡ－ＣＰＧビーズ、

Ｂｚ－ｄＣおよびＩｂｕ－ｄＧによるＩｐｒ－Ｐａｃ－ｄＧ－Ｉｐｒ－Ｐａｃ－ｄＧ－ＣＰＧビーズ、

Ｂｚ－ｄＣおよびＩｂｕ－ｄＧによるＡｃ－ｄＣ－Ａｃ－ｄＣ－ＣＰＧビーズ、

Ｂｚ－ｄＣおよびＩｂｕ－ｄＧによるｄｍｆ－Ｇ－ｄｍｆ－Ｇ－ＣＰＧビーズ、および

上記の４つのオリゴヌクレオチドの混合物。

【００４５】

さらに別の方法では、部分的に脱保護されているオリゴヌクレオチド鎖に保護基を結合してもよい（脱保護手法は以下に詳細に記載されている）。例として以下が挙げられる：

ポリｄＴ２０ｍｅｒｓ（ＤＭＴ基のみ）、

ポリｄＴ２０ｍｅｒｓ（シアノエチル基のみ）、

ポリＩｂｕ－ｄＧ２０ｍｅｒｓ（部分的に脱保護されている）、

ポリＩｐｒ－Ｐａｃ－ｄＧ２０ｍｅｒｓ（部分的に脱保護されている）、

ポリＢｚ－ｄＣ２０ｍｅｒｓ（部分的に脱保護されている）、

ポリＰａｃ－ｄＡ２０ｍｅｒｓ（部分的に脱保護されている）、および

ポリＡｃ－ｄＣ２０ｍｅｒｓ（部分的に脱保護されている）。

【００４６】
本明細書に記載されているように生成される合成オリゴヌクレオチドは以下のように部分的に脱保護することができる：（ａ）合成ポリヌクレオチドに３０％水酸化アンモニウム溶液を添加し、次いで室温において異なる時間インキュベーションし（５、１０および３０分）、（ｂ）処理したオリゴマーのアンモニウム溶液を取り、アンモニウム対酢酸１：４比により１：１希釈し、４℃に事前に冷却した酢酸に添加し、（ｃ）氷浴で３０分試料を維持し、（ｄ）スピード－Ｖａｃで試料を乾燥し、（ｅ）乾燥したペレットを水に溶解し、（ｆ）Ｓｅｐｈａｄｅｘ　Ｇ－２５カラムで試料を脱塩し、（ｇ）スピード－Ｖａｃで試料を乾燥し、（ｈ）脱塩した試料を水に溶解する。

【００４７】
本明細書に記載されているように生成される合成オリゴヌクレオチドは任意の好適な技法によって完全に脱保護することができる。１つの特定の技法は以下のようである：（ａ）合成オリゴヌクレオチドに３０％水酸化アンモニウム溶液を添加し、次いで６５℃において６時間インキュベーションし、（ｂ）スピード－Ｖａｃで試料を乾燥し、（ｃ）乾燥したペレットを水に溶解し、（ｄ）Ｓｅｐｈａｄｅｘ　Ｇ－２５カラムで試料を脱塩し、（ｅ）スピード－Ｖａｃで試料を乾燥し、（ｆ）脱塩した試料を水に溶解する。

【００４８】
部分的および完全に脱保護されたオリゴヌクレオチドは、さらに使用するために、または手法を証明するために、ゲル電気泳動、尿素－アクリルアミドゲル電気泳動、Ｔ４ポリヌクレオチドキナーゼによる５’末端標識、ＨＰＬＣ分析、質量分析法等を含むが、これらに限定されない任意の好適な手段によって、特徴づけることができる。

【００４９】
滅菌生理食塩溶液などの好適な担体にオリゴヌクレオチドを加えたものの非経口注射によって上記のオリゴヌクレオチドで好適な動物を免疫することができる。注射は、皮下、腹腔内、静脈内、動脈内、筋肉内等を含むが、これらに限定されない任意の好適な経路によってもよい。好適な動物は、典型的には、マウス、ウサギ、ラット等を含む哺乳類である。

【００５０】
特定の実施態様において、モノクローナル抗体を作製するためには、若い雌のＢＡＬＢ／ｃマウスを使用し、抗原物質注射の時間経過は以下のようにする：

初日　　最初の注射
１４日め　　最初の追加免疫投与
２８日め　　２回めの追加免疫投与
融合４日前　　最後の追加免疫投与

望ましい場合には、追加の注射を使用してもよい。抗原量は、１回あたり各マウスについて、５０μｇまたは１００μｇの未保護（対照抗体用）または保護オリゴヌクレオチドであってもよい。好ましくは、オリゴヌクレオチド合成の担体として使用されるビーズまたは他の個体担体が動物に注射される場合には、ビーズまたは粒子は水に懸濁され、次いでマウスに注射される。ヌクレオチド溶液を使用する場合には、溶液をフロイントの完全または不完全アジュバントと混合し、マウスに注射する。

【００５１】
ポリクローナル抗体は、既知の技法により上記のように免疫または接種した動物から回収することができ、または既知の技法により、脾臓細胞を動物から回収し、ハイブリドーマ細胞系統を脾臓細胞から作製し、ハイブリドーマ細胞系統を望ましい抗体の作製についてスクリーニングすることができる。

【００５２】
３’または５’末端にビオチン分子を含有するまたは含有しないオリゴヌクレオチド（以下に記載するＥＬＩＳＡアッセイ用）は標準的な技法により合成することができる。例として以下が挙げられる：
ポリＩｂｕ－ｄＧ２０ｍｅｒｓ（ビオチン有または無）

ポリＩｂｕ－ｄＡ２０ｍｅｒｓ（ビオチン有または無）
ポリＩｂｕ－ｄＣ２０ｍｅｒｓ（ビオチン有または無）
ポリＩｐｒ－Ｐａｃ－ｄＧ２０ｍｅｒｓ（ビオチン有または無）
ポリＢｚ－ｄＣ２０ｍｅｒｓ（ビオチン有または無）
ポリＢｚ－ｄＡ２０ｍｅｒｓ（ビオチン有または無）
ポリｄＴ２０ｍｅｒｓ（ビオチン有または無）
ポリＰａｃ－ｄＡ２０ｍｅｒｓ（ビオチン有または無）
ポリＡｃ－ｄＣ２０ｍｅｒｓ（ビオチン有または無）、および
ポリｄｍｆ－Ｇ２０ｍｅｒｓ（ビオチン有または無）。

【００５３】

上記のように作製される抗体は、その結合特性を測定するために、ウェスタンブロットおよびイムノドットーブロットを含むが、これらに限定されない任意の好適な技法によって特徴づけることができる。

【００５４】

ポリクローナル抗体およびモノクローナル抗体を使用する以外に、本発明は、組み換えＤＮＡによる抗体の作製、すなわち「抗体操作」技法を含む。例えば、ハイブリドーマ細胞から単離したｍＲＮＡをｃＤＮＡライブラリーを構築するために使用することができ、抗体全体または抗体断片（例えば、ｓｃＦａｂまたはＦａｂ断片）をコードする配列を単離し、好適な発現ベクターに挿入し、この発現ベクターを、抗体をコードする単離したｃＤＮＡを発現する宿主細胞に挿入することができる。

【００５５】

モノクローナルＦａｂ断片を、当業者に既知の組み換え技法によって大腸菌（Ｅｓｃｈｅｒｉｃｈｉａ　ｃｏｌｉ）中で作製することができる。例えば、Ｗ．Ｈｕｓｅ，Ｓｃｉｅｎｃｅ２４６，１２７５－８１（１９８９）参照。

【００５６】

８．抗体のスクリーニング

保護基特異的抗体についての血清およびハイブリドーマ細胞培養培地のスクリーニングは以下のように実施することができる。

【００５７】

Ａ．血清

１．固体担体に（直接またはオリゴマーを介して）接続した保護基を接種する予定のマウスから免疫前（免疫する前）血清を標準的な手段によって採取する。

２．接種後血清も採取する。

３．特異的な保護基が、マイクロタイタープレートに結合されたビオチン化オリゴヌクレオチドに残存するＥＬＩＳＡアッセイを実施する。他のマイクロタイタープレートウェルは、保護基のない対照オリゴマーまたは他の保護基のオリゴヌクレオチドを含有する。二次抗体は、抗体を可視化するためにホスファターゼがコンジュゲートされているヤギ抗マウスＩｇＧである。

４．特異的な保護基に対して陽性の活性を有するマウスに追加抗原投与し、ハイブリドーマを作製するために犠牲にした。

【００５８】

Ｂ．ハイブリドーマ細胞培養培地

１．約１００の培養物を各脾臓ハイブリッド細胞生成物から作製した。

２．培養物をマイクロタイタープレートウェル、９６ウェルプレートで増殖する。

３．培養培地を各ウェルから除去し、上記のように、～１０００マイクロタイタープレートウェルの各々がプレートに結合された保護されたオリゴヌクレオチドを含有するＥＬＩＳＡアッセイに使用した。

４．陽性の活性を有する抗体を産生する培養物をより大型の培養ウェル、２４ウェルマイクロタイタープレートに移した。

５．大型の培養物の培養培地を、保護基に対する活性について再度試験し、特異性につい

てもアッセイする；すなわち、保護基がないものおよび他の保護基の対照。
６．陽性である培養物を分離（希釈）し、再度試験し、各最終培養が１細胞の結果となる点；すなわち、モノカルチャーまで再度分離した。これらの最終培養物の培地を特異性および親和性について十分に評価する。特異性および親和性はドット－ブロットアッセイを使用して評価する。
【００５９】
Ｃ．ＥＬＩＳＡアッセイの代わりのドット－ブロットアッセイ
１．一部の保護基に対する抗体は、マイクロタイタープレートウェル環境において試験するのに扱い難く、ドット－ブロットアッセイを使用して試験する必要がある。一例は、５’－末端保護基、ジメチル－トリチル（ＤＭＴ）である。
２．ニトロセルロース膜でのドット－ブロットアッセイは、ほとんどの目的のための用途において別の文献に記載されているように実施される。しかし、利用できる培地が少量しかない～１０００マイクロタイターウェル培養物により抗体産生を評価する際にはこれは不可能である。従って、新規改良法が開発された。
ａ）保護されているオリゴヌクレオチドを、ＵＶ－架橋を使用してニトロセルロースにドット状に結合する。ＤＭＴの場合には、膜上の５’－ＤＭＴの存在は弱酸でドットを処理することによって確認され、反転は黄色－橙色に変わる。３’－ビオチンの存在は市販のアビジン染色で確認することができる。
ｂ）膜をブロックする（ドット－ブロットアッセイ参照）。
ｃ）乾燥した膜のドットを慎重に印をつけ（鉛筆）、膜から「くりぬく」。
ｃ）個々のドットを、個々のマイクロタイタープレートウェルの細胞培養培地に加えてインキュベーションする。
ｄ）個々のドットを取り、洗浄し、二次抗体、ホスファターゼ（ｐｈｏｓｐｈｏｔａｓｅ）反応を実施し、適当な試薬を用いたマイクロタイタープレートウェルを使用して呈色させる。
ｅ）陽性のドットを、少量の培養培地を得た元のマイクロタイタープレートウェル培養物に戻って関連させる。
ｆ）さらに培養および分離をＢに記載するように実施する。
【００６０】
９．マイクロアレイの試験
本発明は、マイクロアレイなどの固体支持体に固定したオリゴヌクレオチドを、固体担体で合成したオリゴヌクレオチドの不十分な脱保護または伸長について試験またはスクリーニングするために使用することができる。
【００６１】
本発明を実施するために使用される固体担体は、典型的には、別個の固体担体である。別個の固体担体は物理的に互いに離れていても、または単一構造基板の表面部分の別個の領域であってもよい。このような「チップ型」または「ピン型」固体担体は既知である。例えば、Ｐｉｒｒｕｎｇに付与された米国特許第５，１４３，８５４号、Ｅｌｌｍａｎに付与された米国特許第５，２８８，５１４号（ピン型担体）、Ｆｏｄｏｒらに付与された米国特許第５，５１０，２７０号（チップ型担体）を参照。本発明を実施するために使用することができるオリゴヌクレオチドアレイの別の限定するものではない例およびこれらを製造する方法は、米国特許第５，６３１，７３４号、同第５，５９９，６９５号、同第５，５９３，８３９号、同第５，５７８，８３２号、同第５，５１０，２７０号、同第５，５７１，６３９号、同第６，０５６，９２６号、同第５，４４５，９３４号および同第５，７０３，２２３号に記載されているものを含むが、これらに限定されない。このような装置は、本発明を実施するためにそこに記載されているように使用することができる。
【００６２】
アレイを形成する固体担体または基板は、ケイ素を含む任意の好適な物質を含んでもよい。オリゴヌクレオチドはマイクロアレイ上のｉｎ ｓｉｔｕでモノマー（または個々のヌクレオチド）からｉｎ ｓｉｔｕ重合または成長（ｇｒｏｗｎ）させてもよく（この場合

には、固体担体を分析装置に通過させることができないので、保護基を検出するのに現在利用可能な技法のどれも、アレイ上のオリゴヌクレオチドの不完全な脱保護または伸長を検出するのに有用ではない）、またはオリゴヌクレオチドを別に重合し、次いで固体担体の適当な領域に接続してもよい。アレイは、異なる離れた別個の領域に任意の数の異なるオリゴヌクレオチドを含んでもよく、例として、異なる離れた別個の領域に少なくとも１，０００、少なくとも２，０００、少なくとも１０，０００または少なくとも２０，０００の異なるオリゴヌクレオチドのアレイを含む。

【００６３】

一般に、オリゴヌクレオチドの不十分な脱保護または不十分な伸長についてオリゴヌクレオチドアレイをスクリーニングする方法は以下のステップを含む。

（ａ）上記のオリゴヌクレオチドアレイを提供するステップ、

（ｂ）上記の抗体（すなわち、有機保護基が共有結合した合成オリゴヌクレオチドに特異的に結合し、前記有機保護基が共有結合していない場合には、前記合成オリゴヌクレオチドに結合しない抗体）を提供するステップであって、好ましくは、抗体は、そのアレイが保有するオリゴヌクレオチドの有機合成過程中に保護基が使用される場合に、有機保護基を有するオリゴヌクレオチドに特異的に結合するステップ、次いで

（ｃ）前記抗体に前記オリゴヌクレオチドアレイを接触させ、それによってオリゴヌクレオチドの不十分な脱保護または不十分な伸長の存在を検出するステップであって、定性的であっても、定量的であってもよいこのような検出は上記の任意の好適なイムノアッセイ技法によって実施することができるステップ。

【００６４】

本発明の方法において、ステップ（ｂ）～（ｃ）を、各反復時に異なる抗体を用いて少なくとも１回反復してもよく、その結果、アレイ中のオリゴヌクレオチドに存在することがある複数の異なる保護基を検出することができる。

【００６５】

好ましくは、離れた別個の領域の１つ以上（例えば、複数）のオリゴヌクレオチドの不十分な脱保護（保護基が存在する）が一旦検出されたら、本発明の方法は、アレイの少なくとも１つの離れた別個の位置（または複数の離れた別個の位置）のオリゴヌクレオチドの不十分な脱保護または不十分な伸長の存在を記録する記録または証拠を作成するステップをさらに含む。証拠は、（不十分な伸長を含む）不十分な脱保護の定性的または定量的証拠であってもよい。

【００６６】

上記の方法は、図１１に例示する補正可能なオリゴヌクレオチドアレイを提供する。アレイは、

（ａ）複数の異なるオリゴヌクレオチドが固定された基板３０であって、前記異なるオリゴヌクレオチドが前記基板の異なる離れた別個の位置３１に固定されている基板３０と、

（ｂ）前記アレイに関連する複数の証拠であって、これらの証拠は複数の異なるオリゴヌクレオチドの不十分な脱保護または不十分な伸長の存在を記録し、前記異なるオリゴヌクレオチドは前記アレイの離れた別個の位置に位置づけられている証拠とを組み合わせて含む。これらの証拠は、マイクロリソグラフィーなどの技法によってアレイ３２の領域に印刷されてもよく、紙などの従来の媒体に印刷されて、アレイとともに出荷されてもよく、アレイチップ（位置３２に組み込まれてもよい）に接続されたまたはアレイチップに形成されたメモリーまたは記憶装置に収納されてもよく、フロッピーディスクまたはＣＤ－ＲＯＭなどのコンピュータ読み取り可能な媒体に提供することができる別のデータまたはコンピュータファイルの形態で提供されてもよく、アレイのエンドユーザーによるダウンロードのためにワールドワイドウェブのウェブサイトに格納されてもよい。証拠が別のデータファイルの形態で提供される場合には、アレイは、好ましくは、アレイに形成される、またはアレイに接続されるまたはアレイに関連するコード番号などの識別子（ｉｄｅｎｔｉｆｉｅｒ）をさらに含む（例えば、アレイを含む包装に印刷される、またはアレイと共に包装されるインフォメーションシートに印刷される、および／またはアレイに直接印刷

される）。不十分な脱保護および／または伸長の記録を含む正しい証拠が、アレイの最終的なエンドユーザーによってアレイに最終的に関連づけられることを保証するために、識別子を別の証拠に関連づけることができる（例えば、データシートに印刷する、コンピュータファイルのパスワード、ファイル識別子および／またはアクセスコード等として使用する）。

【００６７】

米国特許第５，９２５，５６２号、同第６，０１７，４９６号、同第５，７５１，６２９号および同第５，７４１，４６２号に記載されているように、アレイに接続したデータ装置または記憶装置を既知の技法により実施することができ、本発明を実施するためにこのような装置をそこに記載されているように使用することができる。

【００６８】

アレイのエンドユーザーは、

（ａ）上記の基板を提供するステップ、

（ｂ）上記の前記アレイに関連する少なくとも１つまたは複数の証拠を提供するステップ、

（ｃ）試験化合物を提供するステップであって、試験化合物は、試験化合物ライブラリーのメンバーであっても、タンパク質、ペプチドまたはオリゴヌクレオチド（例えば、ＤＮＡまたはｍＲＮＡなどのＲＮＡ）などの任意の好適な化合物であってもよいステップ、

（ｄ）（例えば、アレイに試験化合物を接触させることによって）前記複数の異なるオリゴヌクレオチドの少なくとも１つへの前記試験化合物の結合を検出するステップ、次いで、

（ｄ）（ｉ）前記検出された結合、および（ｉｉ）不十分な脱保護または不十分な伸長の存在を記録する前記証拠から、アレイの１つ以上のオリゴヌクレオチドへの試験化合物の結合の程度（単に、結合の有無を含む）を検出し、測定するステップを含む方法において、前記アレイのオリゴヌクレオチドの不十分な脱保護または不十分な伸長を補正するために上記の証拠を使用することができる。従って、アレイの１つ以上の位置のオリゴヌクレオチドの不十分な脱保護または不十分な伸長は、測定ステップ中に補正することができる。このような補正は、アレイの特定の離れた別個の領域を無視することを含む（例えば、同じオリゴヌクレオチドを含有するアレイの他の離れた別個の領域を支持する）、任意の手段によって実施することができる。別の例では、１つ以上の位置が不十分な脱保護または伸長を含有し、それによってそのような位置への結合が減少する場合には、記録されている証拠によって対照を可能にしない場合に示されるものより大きい結合を示すために、そのアレイを用いた実験から誘導される結合データをそれらの位置について上向きに調整することができる。検出または測定ステップは、結合の程度の呈色表示を形成する、結合の程度の数値表示を形成する、結合の程度のグラフまたは他の記号表示を形成する等などの任意の好適な手段によって実施することができる。結合の程度は、結合親和性、結合量、または結合親和性と結合量の両方であるが、典型的には、アレイの特定の離れた別個の領域に結合する試験化合物の量の表示である結合の表示であってもよい。

【００６９】

本発明は、限定することを意図するものではない、以下の実施例においてさらに詳細に説明されている。

【００７０】

［実施例１］

オリゴヌクレオチドの合成

合成は、製造業者のプロトコールに従い、ＡＢＩ　ＤＮＡ／ＲＮＡ　Ｓｙｎｔｈｅｓｉｚｅｒ、モデル３９４（ＰＥ　Ｂｉｏｓｙｓｔｅｍｓ，８５０　Ｌｉｎｃｏｌｎ　Ｃｅｎｔｒｅ　Ｄｒｉｖｅ，Ｆｏｓｔｅｒ　Ｃｉｔｙ，ＣＡ　９４４０４）で実施した。合成中、わずかに改良した１マイクロモルスケールサイクルを使用した（製造業者の取り扱い説明書参照）。主な出発物質（および供給業者／製造業者は括弧内に記載されている）は以下のようであった。

アクティベーター（０．４５Ｍ テトラゾールのアセトニトリル溶液）、ＣＡＰ Ａ（無水酢酸／テトラヒドロフラン／２，６ルチジン），ＣＡＰ Ｂ（Ｎ－メチルイミダゾール／テトラヒドロフラン）および酸化剤（０．０２Ｍヨウ素／ピリジン／ＴＨＦ／Ｈ２Ｏ）（Ｐｒｉｍｅ Ｓｙｎｔｈｅｓｉｓ）
Ｐａｃ－ｄＡ（５’－ジメトキシトリチル－Ｎ－フェノキシアセチル－２’－デオキシアデノシン、３’－［（２－シアノエチル）－（Ｎ，Ｎ－ジイソプロピル）］－ホスホールアミダイト（Ｇｌｅｎ Ｒｅｓｅａｒｃｈ）
Ｉｐｒ－Ｐａｃ－ｄＧ（５’－ジメトキシトリチル－Ｎ－ｐ－イソプロピル－フェノキシアセチル－２’－グアノシン、３’－［（２－シアノエチル）－（Ｎ，Ｎ－ジイソプロピル）］－ホスホールアミダイト（Ｇｌｅｎ Ｒｅｓｅａｒｃｈ）
Ａｃ－ｄＣ（５’－ジメトキシトリチル－アセチル－２’－デオキシシチジン、３’－［（２－シアノエチル）－（Ｎ，Ｎ－ジイソプロピル）］－ホスホールアミダイト（Ｇｌｅｎ Ｒｅｓｅａｒｃｈ）
ｄｍｆ－Ｇ（５’－ジメトキシトリチル－ジメチルホルムアミジン－グアノシン、２’－Ｏ－ＴＢＤＭＳ－３’－［（２－シアノエチル）－（Ｎ，Ｎ－ジイソプロピル）］－ホスホールアミダイト（Ｇｌｅｎ Ｒｅｓｅａｒｃｈ）
Ｂｚ－ｄＣ－ＣＰＧビーズ（５’－ジメトキシトリチル－Ｎ－ベンゾイル－２’－デオキシシチジン、３’－［（２－シアノエチル）－（Ｎ，Ｎ－ジイソプロピル）］－ホスホールアミダイト－スクシニルリンカー－ビーズ（３０００Ａｎｇ）（ＣＰＧ Ｉｎｃ．）
Ｉｂｕ－ｄＧ－ＣＰＧビーズ（５’－ジメトキシトリチル－Ｎ－イソブチル－２’－デオキシシチジン、３’－［（２－シアノエチル）－（Ｎ，Ｎ－ジイソプロピル）］－ホスホールアミダイト－スクシニルリンカー－ビーズ（３０００Ａｎｇ）（ＣＰＧ Ｉｎｃ．）

【００７１】

以下の化合物を合成した。化合物は以下に示すビーズに結合した：
Ｐａｃ－ｄＡ－Ｐａｃ－ｄＡ－Ｂｚ－ｄＣ－スクシニルリンカー－ビーズ
Ｐａｃ－ｄＡ－Ｐａｃ－ｄＡ－Ｉｂｕ－ｄＧ－スクシニルリンカー－ビーズ
Ｉｐｒ－Ｐａｃ－ｄＧ－Ｉｐｒ－Ｐａｃ－ｄＧ－Ｂｚ－ｄＣ－スクシニルリンカー－ビーズ
Ｉｐｒ－Ｐａｃ－ｄＧ－Ｉｐｒ－Ｐａｃ－ｄＧ－Ｉｂｕ－ｄＧ－スクシニルリンカー－ビーズ
Ａｃ－ｄＣ－Ａｃ－ｄＣ－Ｂｚ－ｄＣ－スクシニルリンカー－ビーズ
Ａｃ－ｄＣ－Ａｃ－ｄＣ－Ｉｂｕ－ｄＧ－スクシニルリンカー－ビーズ
ｄｍｆ－Ｇ－ｄｍｆ－Ｇ－Ｂｚ－ｄＣ－スクシニルリンカー－ビーズ
ｄｍｆ－Ｇ－ｄｍｆ－Ｇ－Ｉｂｕ－ｄＧ－スクシニルリンカー－ビーズ。

【００７２】

上記の化合物は、以下の実施例２にさらに記載されているように、抗体を作製するために、オリゴヌクレオチドを固体担体から分離することなく、免疫原として動物に直接投与した。

【００７３】

［実施例２］

動物の接種

８～１２週齢の雌ＢＡＬＢ／ｃマウスをＣｈａｒｌｅｓ Ｒｉｖｅｒ，Ｒａｌｅｉｇｈ，Ｎｏｒｔｈ Ｃａｒｏｌｉｎａ，ＵＳＡから購入した。マウスは、フィルターキャップ付きのケージで飼育した。

【００７４】

オリゴヌクレオチド鎖の合成を実施例１に記載されているように実施したら、ガラス板の間にビーズを置いて、ガラス板を手で押してヌクレオチド付きビーズをやさしく粉砕した。

【００７５】

上記の８つのオリゴヌクレオチドの各々５μＭを４ｍｌのＰＢＳ（１５０ｍＭ塩化ナトリ

ウムを１００ｍＭリン酸緩衝液に加えたもの、ｐＨ７．２）に混合した。
【００７６】
混合物を十分に撹拌して、粉砕したビーズを懸濁させた。撹拌した混合物１５０μＬを取り、注射筒のＰＢＳ３００μＬに添加した。注射直前に、破壊したビーズを懸濁させるために、注射筒を振とうすることによって、ビーズを含有する溶液を再度混合した。次いで、十分に混合した溶液の１５０μＬまたは３００μＬをマウスの腹腔内に注射した。この手法を最初の注射と以下の追加抗原投与に使用した。
注射時間スケジュール

注射	日数（日）
１回め	０
２回め	１４日め
３回め	２８日め
４回め	４２日め
５回め	５６日め
６回め	７０日め
７回め	８４日め
８回め	９８日め
９回め	１１２日め
１０回め	１３８日め
１１回め（最後、融合の４日前）	１４２日め

【００７７】
最後の注射の４日後、脾臓細胞を動物から採取し、ハイブリドーマ細胞系統を作製するために既知の技法により骨髄腫細胞（Ｐ３ｘ．６３．Ａｇ８．６５３）と融合し、次いで特定の細胞系統を単離し、本発明の望ましい抗体を作製するために、以下に記載する特徴であるかどうかを判定するためにスクリーニングした。
【００７８】
［実施例３］
抗体の特徴づけのためのイムノドット－ブロットアッセイ
イムノドット－ブロットアッセイは膜の紙へのオリゴヌクレオチドのＵＶ架橋に関係し、生成物オリゴマーの保護基を検出、同定および定量するための試験キットに直接適用することができる。この手法は以下のように実施することができる：（ａ）ＴＢＳ（１０ｍＭ　Ｔｒｉｓ，ｐＨ７．２；１５０ｍＭ　ＮａＣｌ）で膜の紙を濡らす、（ｂ）減圧下で膜の紙に試験対象のオリゴヌクレオチドをドットする、（ｃ）膜の紙にヌクレオチドをＵＶ架橋する、（ｄ）室温において２時間または４℃において終夜１％カゼイン－ＴＢＳＴ（ＴＢＳプラスＴｗｅｅｎ２０，０．１容量％）で膜の紙をブロックする、（ｅ）ＴＢＳＴで各々１５分ずつ３回膜を洗浄する、（ｆ）室温においてプレートを試験対象の試料（１％カゼイン－ＴＢＳＴで希釈）と共に１時間インキュベーションすることによって抗原－抗体複合体を形成する、（ｇ）上記のように洗浄する、（ｈ）室温において１時間第２抗体接合体（１％カゼイン－ＴＢＳＴで希釈）と反応させる、（ｉ）上記のように洗浄する、（ｊ）膜を基板溶液と共にインキュベーションすることによって呈色反応を形成する。
【００７９】
［実施例４］
モノクローナル抗体１Ｈ１１のドット－ブロットアッセイ
上記の実施例２に記載するように作製したモノクローナル抗体１Ｈ１１をドット－ブロットアッセイによって特徴づけた。結果は、図１の棒グラフとして示す。図１において、レーン（またはカラム）１および２は、それぞれ、６５℃において６時間および４℃において１５分間NH_4OHで処理したオリゴＰａｃ－ｄＡ２０ｍｅｒｓを示す。カラム３および４は、それぞれ、６５℃において６時間および１５分間NH_4OHで処理したオリゴＢｚ－ｄＣ２０ｍｅｒｓを示す。カラム５および６は、それぞれ、６５℃において６時間および１５分間NH_4OHで処理したオリゴＡｃ－ｄＣ２０ｍｅｒｓを示す。カラム７およ

び８は、それぞれ、６５℃において６時間および１５分間NH_4OHで処理したオリゴＩｐｒ－Ｐａｃ－ｄＧ２０ｍｅｒｓを示す。カラム９および１０は、それぞれ、６５℃において６時間および１５分間NH_4OHで処理したオリゴＩｂｕ－ｄＧ２０ｍｅｒｓを示す。カラム１１、１２および１３は、それぞれ、ＤＭＴ基だけおよびシアノエチル基だけを有する完全に脱保護されているオリゴｄＴ２０ｍｅｒｓを示す。抗体活性はＥＬＩＳＡの光学密度（４７９ｎｍ）として示し（以下の実施例７）、ドット－ブロットアッセイの陽性または陰性の結果は、棒グラフの各カラムの上方の白丸または黒丸で示してある。モノクローナル抗体１Ｈ１１の活性はカラム１０ではオリゴＩｂｕ－ｄＧ２０ｍｅｒに選択的に結合していることに注目すべきである。

【００８０】

［実施例５］

モノクローナル抗体７Ｈ３のドット－ブロットアッセイ

上記の実施例２に記載するように作製したモノクローナル抗体７Ｈ３を、上記の実施例３に記載するドット－ブロットアッセイによって特徴づけた。結果は、図１の棒グラフとして示す。図１において、レーン（またはカラム）１および２は、それぞれ、６５℃において６時間および４℃において１５分間NH_4OHで処理したオリゴＰａｃ－ｄＡ２０ｍｅｒｓを示す。カラム３および４は、それぞれ、６５℃において６時間および１５分間NH_4OHで処理したオリゴＢｚ－ｄＣ２０ｍｅｒｓを示す。カラム５および６は、それぞれ、６５℃において６時間および１５分間NH_4OHで処理したオリゴＡｃ－ｄＣ２０ｍｅｒｓを示す。カラム７および８は、それぞれ、６５℃において６時間および１５分間NH_4OHで処理したオリゴＩｐｒ－Ｐａｃ－ｄＧ２０ｍｅｒｓを示す。カラム９および１０は、それぞれ、６５℃において６時間および１５分間NH_4OHで処理したオリゴＩｂｕ－ｄＧ２０ｍｅｒｓを示す。カラム１１、１２および１３は、それぞれ、ＤＭＴ基だけおよびシアノエチル基だけを有する完全に脱保護されているオリゴｄＴ２０ｍｅｒｓを示す。抗体活性は上記の光学密度として示し、ドット－ブロットアッセイの陽性または陰性の結果は、棒グラフの各カラムの上方の白丸または黒丸で示してある。モノクローナル抗体１Ｈ１１の活性はカラム４ではオリゴＢｚ－ｄＣ２０ｍｅｒに選択的に結合していることに注目すべきである。

【００８１】

［実施例６］

抗体を特徴づけるためのウェスタンブロットアッセイ

ウェスタンブロットアッセイは、オリゴヌクレオチドのゲルから膜の紙への低電圧移動およびオリゴヌクレオチドの膜へのＵＶ架橋に関係する。このアッセイは以下のように実施することができる：（ａ）１０ｍＭＭｇCl_2を含有する１５％未変性ゲルを注ぐ、（ｂ）ゲルのウェルにオリゴヌクレオチド（オリゴマー）をロードする、（ｃ）氷浴中で２００ボルトでゲルを泳動する、（ｄ）氷浴中で２５ボルトで２５分間ゲルから膜の紙にオリゴヌクレオチドを移動させる、（ｅ）膜にヌクレオチドをＵＶ架橋する、（ｆ）室温において２時間または４℃において終夜１％カゼイン－ＴＢＳＴで膜の紙をブロックする、（ｇ）ＴＢＳＴで各々１５分ずつ３回膜を洗浄する、（ｈ）室温において試験対象の試料（１％カゼイン－ＴＢＳＴで希釈）を１時間インキュベーションする、（ｉ）上記のように洗浄する、（ｊ）室温において１時間膜を第２抗体接合体（１％カゼイン－ＴＢＳＴで希釈）と共にインキュベーションする、（ｋ）上記のように洗浄する、（ｌ）膜を基板溶液と共にインキュベーションすることによって呈色を形成する。

【００８２】

［実施例７］

抗原としてビオチン化ポリヌクレオチドを使用した抗体の検出およびストレプトアビジン－ビオチン系に関係するＥＬＩＳＡ

抗体を検出するための酵素結合免疫吸着アッセイ（ＥＬＩＳＡ）は以下のように実施する：（ａ）ストレプトアビジンを事前にコーティングしたマイクロタイタープレートを事前スクリーニングする、（ｂ）試験対象のビオチン化オリゴヌクレオチドまたは他の物質（

濃度５μｇ／ｍｌのＰＢＳ溶液）（ＰＢＳ：１５ｍＭ　ＮａＣｌ，１０ｍＭ　リン酸緩衝液、ｐＨ７．４）の調製物でプレートをコーティングし、次いで室温において２時間インキュベーションする、（ｃ）０．１％ＴｗｅｅｎのＰＢＳ溶液（ＰＢＳＴ）で各々１５分ずつ３回を洗浄する、（ｄ）室温において時間または４℃において終夜１％カゼインのＰＢＳＴ溶液でブロックする、（ｅ）上記のように洗浄する、（ｆ）プレートを抗体と共に室温において１時間インキュベーションすることによって抗原－抗体複合体を形成する、（ｇ）上記のように洗浄する、（ｆ）室温において１時間第２抗体－ペルオキシダーゼ接合体（１％カゼイン－ＰＢＳＴ溶液）と反応させる、（ｉ）上記のように洗浄する、（ｊ）テトラメチルベンジジン（ＴＭＢ）溶液（ＴＭＢ溶液：４２ｍＭ　ＴＭＢ、０．００４％H_2O_2、０．１Ｍ酢酸緩衝液、ｐＨ５．６）を添加し、室温において１５分間インキュベーションし、次いで２ＭH_2SO_4で反応を停止することによって呈色反応を形成する、（ｋ）４６９ｎｍの吸光度値を読む。

【００８３】

［実施例８］

ベンゾイル、イソブチリルおよびイソプロピルフェノキシアセチルに対するモノクローナル抗体のＥＬＩＳＡおよびドット－ブロットアッセイ

上記の実施例２に記載するように作製した、保護基ベンゾイル（Ｂｚ）、イソブチリル（ｉｂｕ）およびイソプロピルフェノキシアセチル（ｉｐｒ－Ｐａｃ）に対するモノクローナル抗体（ｍＡｂ）を、上記の実施例３に記載する、標準的なＥＬＩＳＡアッセイおよびドット－ブロットアッセイによって特徴づけた。９６－ウェルマイクロタイタープレートに各々結合した２０残基のビオチン化核酸で展開したＥＬＩＳＡアッセイは、それぞれの抗原に対する抗体の特異性を証明した。図３Ａ、図４Ａおよび図５Ａは、それぞれ、Ｂｚ、ｉｂｕおよびｉｐｒ－Ｐａｃに対するモノクローナル抗体の結果を示す。図は、オリゴｄＣ（Ｂｚ）と名づけられる、すなわち、最初はＢｚで保護されていた、完全に脱保護されている（＜１％Ｂｚ残存）ｄＣ残基のホモポリマー（レーン１、白ぬきの棒）、保護されている（＞９７％Ｂｚ残存）オリゴＢｚ－ｄＣ（レーン２、影つきの棒）、完全に（＜１％ｉｐｒ－Ｐａｃ残存）脱保護されているオリゴｄＧ（ｉｐｒ－Ｐａｃ）（レーン３）、保護されている（＞７６％ｉｐｒ－Ｐａｃ）オリゴｉｐｒ－ＰａｃｄＧ（レーン４）、完全に（＜１％ｉｂｕ残存）脱保護されているオリゴｄＧ（ｉｂｕ）（レーン５）、保護されている（＞９１％ｉｂｕ残存）オリゴｉｂｕ－ｄＧ（レーン６）および完全に脱保護されているオリゴｄＴ（レーン７）を示す。ｄＴポリマーは、１つの保護基以外に、弱酸で５’－末端残基の５’ＯＨから除去されるジメチルトリチル（ＤＭＴ）を有した。最後にレーン８は、ＤＭＴが残存するオリゴｄＴを示す。

【００８４】

２０ｍｅｒのＤＮＡｓをニトロセルロース膜にＵＶで結合した、抗ＢｚｍＡｂ、抗ｉｂｕｍＡｂおよび抗ｉｐｒ－ＰａｃｍＡｂのドット－ブロットアッセイを実施した。膜に適用した２０ｍｅｒＤＮＡの量を図３Ｂ、図４Ｂおよび図５Ｂの右に示し、アッセイ感度のレベルを証明する。抗ＢｚｍＡｂを試験するために使用したＤＮＡｓは、ＥＬＩＳＡについて記載したものプラス脱保護されているオリゴｄＡ（Ｂｚ）、保護されているオリゴＢｚ－ｄＡ、オリゴｄＣ（ｉｂｕ）、オリゴｉｂｕ－ｄＣ、オリゴｄＡ（ｉｂｕ）およびオリゴｉｂｕ－ｄＡであった。図３Ｂは、抗ＢｚｍＡｂはｄＡおよびｄＣの保護基を認識したことを示している。抗ｉｂｕｍＡｂを試験するために使用したＤＮＡｓは、ＥＬＩＳＡについて記載したものプラス保護されているオリゴｉｂｕ－ｄＡ、脱保護されているオリゴｄＡ（ｉｂｕ）、オリゴｉｂｕ－ｄＣ、オリゴｄＣ（ｉｂｕ）であり、全てドット－ブロットの上部に記載されている。図４Ｂは、抗ｉｂｕｍＡｂは、保護基のうち最も一般的な用途の、ｄＧのｉｂｕを認識したが、ｄＡでも認識したことを示している。抗ｉｐｒ－ＰａｃｍＡｂを試験するために使用したＤＮＡｓは、ＥＬＩＳＡについて記載したものプラス保護されているオリゴｉｂｕ－ｄＡ、脱保護されているオリゴｄＡ（ｉｂｕ）、オリゴｉｂｕ－ｄＣ（ｉｂｕ）、オリゴＢｚ　ｄＡ（Ｂｚ）であり、全てドット－ブロットの上部に記載されている。図５Ｂは、抗ｉｐｒ－ＰａｃｍＡｂは、保護基のうち最も一般的な

用途の、ｄＧのｉｐｒ－Ｐａｃを認識したが、ｄＡおよびｄＣでも認識したことを示している。ｍＡｂはまたｉｂｕ保護基を認識した（ｉｂｕ－ｄＧ、ｉｂｕ－ｄＡおよびｉｂｕ－ｄＣ）。この交差反応は、抗体は、ｉｐｒ－Ｐａｃおよびｉｂｕに共通の化学、おそらくＣＨ（CH_3）$_2$の識別に選択性が高いことを示している。従って、抗ｉｂｕおよび抗ｉｐｒ－ＰａｃｍＡｂｓは、オリゴに残存する保護基を同定するために組み合わせて使用することができると思われる。

【００８５】

抗ｉｂｕｍＡｂのドット－ブロットアッセイには大量のＤＮＡを試験した（図４Ｃ）。この実験の結果は、ｉｂｕ保護基は、どの核塩基が保護されているかにかかわらず、ｍＡｂによって認識されることを証明した。

【００８６】

図３Ｃ、図４Ｃおよび図５Ｃは、部分的に脱保護されたオリゴマーを再処理して、残存する保護基を除去し、ｍＡｂで再試験することができることを証明している。図３Ｃは、抗ＢｚｍＡｂは再度脱保護されたオリゴマーオリゴＢｚ－ｄＣを認識したことを示している（中央のカラム）。同様に、図４Ｄは、抗ｉｂｕｍＡｂは再度脱保護されたオリゴマーオリゴｉｂｕ－ｄＧ（中央のカラム）を認識することを示し、図５Ｃは、抗ｉｐｒ－ＰａｃｍＡｂは再度脱保護されたオリゴマーオリゴｉｐｒ－Ｐａｃ－ｄＧおよびオリゴｉｂｕ－ｄＧ（それぞれ、左から２番めおよび４番めのカラム）を認識することを示している。従って、この方法は、高価な核酸試料を破棄する必要なく、品質管理に適用可能である。

【００８７】

保護基Ｂｚ、ｉｂｕおよびｉｐｒ－Ｐａｃを有するＲＮＡ標品を合成し、Ｂｚ（図３Ｄ）、ｉｂｕ（図４Ｅ）およびｉｐｒ－Ｐａｃ（図５Ｄ）に対するｍＡｂを用いた保護基の同定についてアッセイした。ドット－ブロットアッセイは、モノクローナル抗体はＲＮＡとＤＮＡを識別しないことを明らかに示している。ＲＮＡにはＤＮＡより高いバックグラウンドシグナルが存在したが、特に少量のＲＮＡでは保護基のあるＲＮＡと保護基のないＲＮＡには有意な差があった。膜のＲＮＡの量は試料の吸光度から推定した。

【００８８】

［実施例９］

保護基のｍＡｂドット－ブロットアッセイとＨＰＬＣ

標準化した２０ｍｅｒのオリゴｄＣ分子に残存するＢｚ基のドット－ブロット検出を実施例３に記載するように実施した。完全に脱保護されたｄＣ２０ｍｅｒと未処理のｄＣ２０ｍｅｒを、全く独立した異なる定量方法を使用して分析した。２つのオリゴマーを構成ヌクレオシドに加水分解し、濃縮した試料を用いた認識されている高速液体クロマトグラフィー（ＨＰＬＣ）を使用して同定し、定量した。感度が悪いので、ＨＰＬＣ検出は、ｍＡｂアッセイに使用されるＢｚ－ｄＣの量の５０～１００倍が必要であった（図７参照）。図６Ａは、保護されているオリゴＢｚ－ｄＣのｎｍｏｌｅ量のＢｚ基に対して試験した抗ＢｚｍＡｂの結果（右のカラム）およびＢｚ－ｄＣの同じｎｍｏｌｅ量のＢｚ－の結果（左のカラム）示す。各量のＢｚ－ｄＣオリゴを同じ鎖長（２０ｍｅｒ）の完全に脱保護されているｄＣオリゴで希釈して、２５００倍のｄＣが存在する場合でも（すなわち、０．０４％）ｍＡｂの検出感度があることを証明した。ｍＡｂアッセイは、ｍＡｂは、２５００倍過剰量のｄＣがＤＮＡ中に存在する場合でも、ＤＮＡのＢｚ基を検出することができることを証明した。

【００８９】

ｍＡｂ応答を定量するために、図６Ａに示すドット－ブロットに濃度測定を実施した。バックグラウンドを引いた後、ＨＰＬＣで測定したオリゴＢｚ－ｄＣのＢｚ基の関数として残存する密度をプロットした（図６Ｂ）。データは、抗ＢｚｍＡｂ検出の高感度は０．１～１．０ｎｍｏｌの範囲において直線状であることを示した。

【００９０】

次に、ｍＡｂ応答が、ドット－ブロット膜のＤＮＡ量の増加に伴って増加するかどうかを測定した。Ｂｚの量は標準的なＨＰＬＣ方法で測定した。保護されている試料と脱保護さ

れている試料の１／２５００の比の混合物中のＢｚ保護基の検出は、膜のＤＮＡの量を増加することによって増加するが、比は維持されることをこの実験は示した（図６Ｃ）。

【００９１】

最後に、ＢｚのｍＡｂ検出とＨＰＬＣ検出の直接比較を示すために、実験を実施した。オリゴＢｚ－ｄＣ（２０ｍｅｒ）のｄＣのＢｚを検出するために抗ＢｚｍＡｂをドット－ブロットアッセイに使用した。ｍＡｂアッセイにより検出され、濃度測定によって定量されたＢｚ基の密度応答を、各ドットのＤＮＡのＢｚの量に対してプロットした（図７Ａ）。ＤＮＡ中のＢｚの量は、大量のＤＮＡの消化並びにＢｚ－ｄＣモノヌクレオシドのＨＰＬＣ同定および定量による分析によって較正した。ＨＰＬＣ実験では、３つのＢｚ－ｄＣオリゴ試料を加水分解し、ＨＰＬＣによって組成を分析した。ＵＶ－ダイオードアレイディテクターの応答を試料中のＢｚの量に対してプロットした（図７Ｂ）。試料の量は、既知量のＢｚ－ｄＣで「ピークを生じた（ｓｐｉｋｅｄ）」試料と比較することによって求めた。スパイク（ｓｐｉｋｅ）として試料に添加したＢｚ－ｄＣの量は、秤量したＢｚ－ｄＣストックによるものであった。従って、ＨＰＬＣ応答は、既知量のＢｚ－ｄＣを用いて較正した。これらの実験の結果は、抗ＢｚｍＡｂによるＢｚの検出はｐｍｏｌｅ範囲内であるが、ＢｚのＨＰＬＣ検出はｎｍｏｌｅ範囲に制限されることを示している。

【００９２】

［実施例１０］

市販の試料中の残存保護基の検出

市販の試料中の残存保護基のｍＡｂによる検出を証明するために盲検検討を実施した。この実験の目的は、オリゴ合成業界において通常実施されているように処理されたおそらく完全に脱保護されている試料においてｍＡｂ技術で保護基を検出および同定できるかどうかを判定することであった。選択した８社によって使用されている保護基の性質は未知であったので、実験は盲検試験であった。８社の各々製の２つの２０ｍｅｒオリゴ（オリゴｄＡ－ｄＣおよびオリゴｄＧ－ｄＴ）を合成して、脱保護するように注文し、できるだけ理想的な条件下で塩を除去した。オリゴは通常どおり速達で出荷され、ドット－ブロットによるｍＡｂ分析を実施した。１社（＃６）とおそらくもう１社（＃２）製のｄＡ－ｄＣオリゴは、抗ＢｚｍＡｂ試験によって測定したとき、Ｂｚ保護基が残存していた（図８Ａ）。２社（＃２および＃６）製のｄＧ－ｄＴオリゴは、抗ｉｐｒ－ＰａｃｍＡｂで測定したとき、ｉｐｒ－Ｐａｃ保護基が残存していた（図８Ｂ）。市販の試料中の残存保護基は、試料の量を増加し、さらに脱保護して再分析することによって確認した。＃２社および＃６社製のオリゴｄＡ－ｄＣ試料は、Ｂｚ保護基の存在を確認するためにより大量で試験した。また、標準的なプロトコールを使用して、残存する保護基を除去するために試料を処理した。さらに脱保護した後の再分析は、基は今度は除去されたことを示した（図８Ｃ）。これは、高価な核酸試料を再処理して保護基を除去すると、それらを破棄する必要がないことを証明している。オリゴｄＧ－ｄＴ試料を再処理して、残存する保護基を除去し、抗ｉｐｒ－ＰａｃｍＡｂで再分析すると、ｉｐｒ－Ｐａｃ基はＤＮＡを犠牲にすることなく除去されうるという結果が得られた（図８Ｄ）。

【００９３】

ジメトリチルに対するポリクローナル抗体

５’末端保護基、ジメトリチル（ＤＭＴ）に対するポリクローナル抗体の作製および分析は実施例２に記載されているとおりであった。４匹のマウスにＤＭＴを接種し、数週間抗原で追加免疫投与してから、血清をマウスから採取した。ＤＭＴ［ＤＭＴ－ＯＨ］、デオキシヌクレオチドトリマーｄ（Ｔ）$_3$［（ＤＭＴ）$_3$－ｄ（Ｔ）$_3$］の５’－末端の３つのＤＭＴ、３’－ビオチンを有するデオキシヌクレオチド２０ｍｅｒ［（ＤＭＴ）$_3$－ｄ（Ｔ）$_{20}$－ビオチン］の５’－末端の３つのＤＭＴ、３’－ビオチンを有するデオキシヌクレオチド２０ｍｅｒ［ＤＭＴ－ｄ（Ｔ）$_{20}$－ビオチン］の５’－末端の１つのＤＭＴ、３’－ビオチンを有するｄＴ２０ｍｅｒ［ｄ（Ｔ）$_{20}$－ビオチン］，ビオチンを有するＤＭＴ［ＤＭＴ－ビオチン］の１つのＤＭＴおよびトリス－ボレート生理食塩液対照をニトロセルロース膜に適用し、次いで抗ＤＭＴ抗体を評価するためのマウス血清（接種

マウス＃１～４および対照血清、正常）、ＤＭＴの存在をあきらかにするための弱酸（ＴＢＳ）およびビオチンの存在を明らかにするためのアビジンを用いてアッセイした（図９）。マウス＃２および＃４の血清はＤＭＴ［（ＤＭＴ）$_3$－ｄ（Ｔ）$_3$として］を認識したが、＃１、＃３および正常なマウスは認識しなかった。弱酸は黄色としてＤＭＴの存在を明らかにし（図には示していない）、アビジンはビオチンの存在を明らかにした。

【００９４】

上記は本発明を例示するものであり、本発明を限定するものと解釈されるべきではない。本発明は、以下の請求の範囲およびそこに含まれる請求の範囲の等価物によって規定される。

【図面の簡単な説明】

【図１】

オリゴＩｂｕ－ｄＧ２０ｍｅｒｓに選択的に結合する、モノクローナル抗体１Ｈ１１のドット－ブロットイムノアッセイである。

【図２】

オリゴＢｚ－ｄＣ２０ｍｅｒｓに選択的に結合する、モノクローナル抗体７Ｈ３のドット－ブロットイムノアッセイである。

【図３】

核酸の化学合成に通常使用される保護基、ベンゾイル（Ｂｚ）のモノクローナル抗体（ｍＡｂ）の特異性および検出感度を証明するＥＬＩＳＡ（Ａ）およびドット－ブロット（Ｂ）を示す。部分的に脱保護されたオリゴマーオリゴＢｚ－ｄＣ（中央のカラム）を再処理して、残存する保護基を除去し、ｍＡｂで再試験することができる（Ｃ）。保護基Ｂｚ、ｉｂｕおよびｉｐｒ－Ｐａｃを有するＲＮＡ標品を合成し、Ｂｚに対するｍＡｂによる保護基の同定をアッセイした（Ｄ）。

【図４】

核酸の化学合成に通常使用される保護基、イソブトリル（ｉｂｕ）のモノクローナル抗体（ｍＡｂ）の特異性および感度並びにその検出を証明するＥＬＩＳＡ（Ａ）およびドット－ブロット（Ｂ）結果を示す。大量のＤＮＡを用いたドット－ブロットアッセイは、ｉｂｕ保護基は、どの核酸塩基が保護されているかにかかわらず、ｍＡｂによって認識されたことを証明している（Ｃ）。部分的に脱保護されたオリゴマーオリゴＢｚ－ｄＣ（中央のカラム）を再処理して、残存する保護基を除去し、ｍＡｂで再試験することができる（Ｄ）。保護基Ｂｚ、ｉｂｕおよびｉｐｒ－Ｐａｃを有するＲＮＡ標品を合成し、ｉｂｕに対するｍＡｂによる保護基の同定をアッセイした（Ｅ）。

【図５】

核酸の化学合成に通常使用される保護基、イソプロピルフェノキシアセチル（ｉｐｒ－Ｐａｃ）のモノクローナル抗体（ｍＡｂ）の特異性および感度並びにその検出を証明するＥＬＩＳＡ（Ａ）およびドット－ブロット（Ｂ）結果を示す。部分的に脱保護されたオリゴマーオリゴｉｐｒ－Ｐａｃ－ｄＧおよびオリゴｉｂｕ－ｄＧ（それぞれ、左から２番目および４番目のカラム）を再処理して、残存する保護基を除去し、ｍＡｂで再試験することができる（Ｃ）。保護基Ｂｚ、ｉｂｕおよびｉｐｒ－Ｐａｃを有するＲＮＡ標品を合成し、ｉｐｒ－Ｐａｃに対するｍＡｂによる保護基の同定をアッセイした（Ｄ）。

【図６】

ＨＰＬＣに関連する技術の感度および定量応答を証明する、保護基のｍＡｂドット－ブロットアッセイを示す。標準の２０ｍｅｒオリゴｄＣ分子に残存するＢｚ基のドット－ブロット検出を実施し（Ａ）、ｍＡｂ応答の定量（Ｂ）を行った。ｍＡｂ応答は、ドット－ブロット膜のＤＮＡ量を増加させて分析した（Ｃ）。左のカラムは保護されているＢｚ－ｄＣ２０ｍｅｒである。右のカラムは、保護されているＢｚ－ｄＣと２５００倍過剰量の完全に脱保護されたオリゴｄＣ（Ｂｚ）である。

【図７】

それぞれ、ｐｍｏｌｅ（Ａ）およびｎｍｏｌ範囲（Ｂ）のＢｚのｍＡｂおよびＨＰＬＣ検出の直接比較を示す。

【図８】
市販の試料中に残存する保護基の検出を証明する盲検試験を示す。ｄＡ－ｄＣオリゴは抗ＢｚｍＡｂ（Ａ）で分析し、ｄＧ－ｄＴオリゴは抗ｉｐｒ－ＰａｃｍＡｂ（Ｂ）で分析した。＃２社および＃６社製のオリゴｄＡ－ｄＣ試料は、Ｂｚ保護基の存在を確認するために大量で試験した（Ｃ）。また、標準的なプロトコールを使用して残存する保護基を除去するために試料を処理した。オリゴｄＧ－ｄＴ試料はｉｐｒ－Ｐａｃ保護基についてアッセイした（Ｄ）。試料を再処理して、残存する保護基を除去し、（Ｃ）のように再分析した。
【図９】
５’末端保護基、ジメトリチル（ＤＭＴ）に対するポリクローナル抗体の作製および分析を示す。
【図１０】
配列は同じで類似したオリゴヌクレオチドを、保護または脱保護の程度の差についてスクリーニングする試験標準として使用することができる、配列が同じで、脱保護の程度が異なる種々のオリゴヌクレオチドを含有する基板を示す。
【図１１】
本発明の抗体を用いて、保護基の存在または不十分な伸長をスクリーニングすることができるオリゴヌクレオチドアレイを例示する。

【図１】

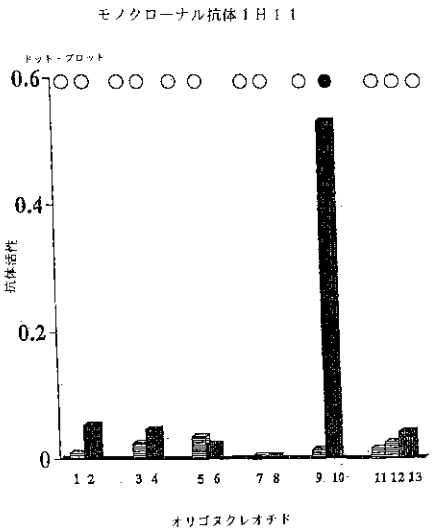


FIG 1

【図２】

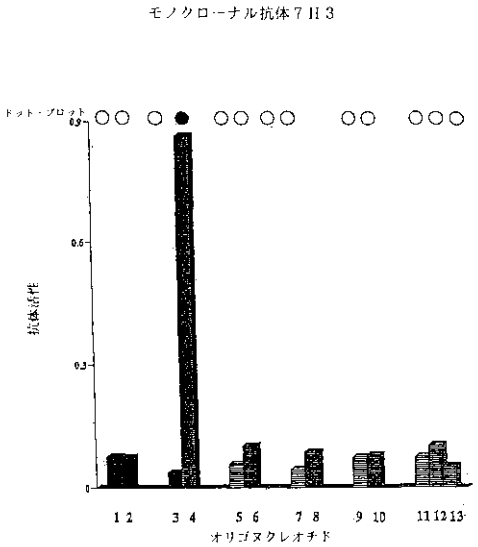


FIG 2

【図３】

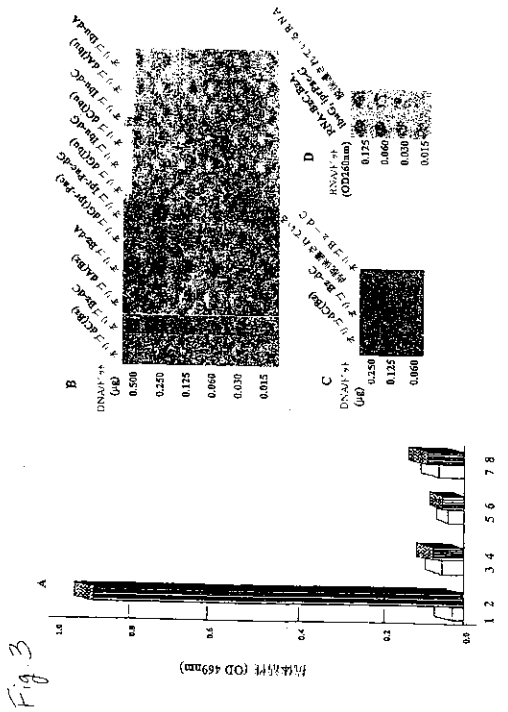


Fig.3

【図４】

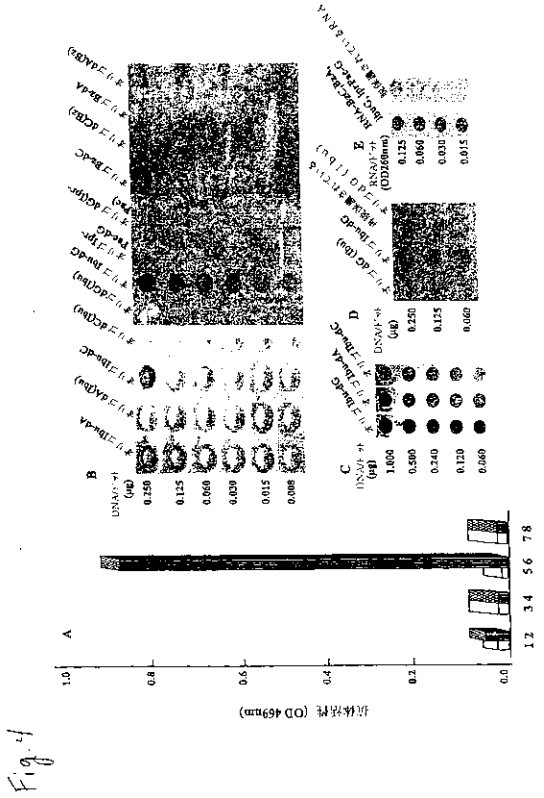


Fig.4

【図５】

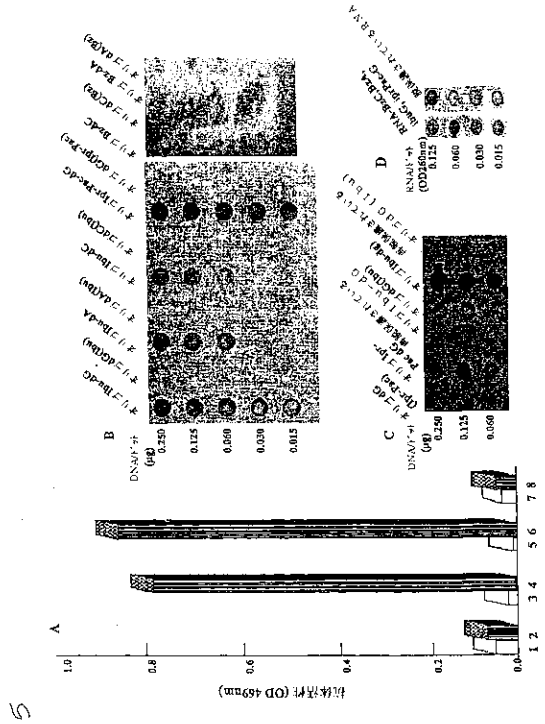


Fig.5

【図６】

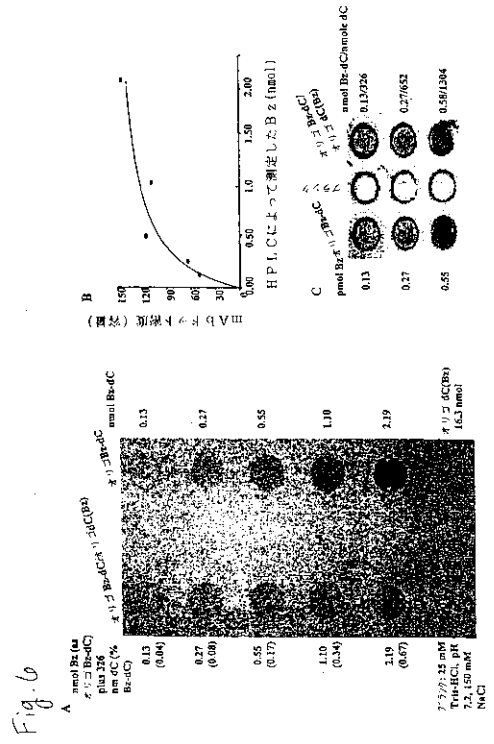


Fig.6

【図７】

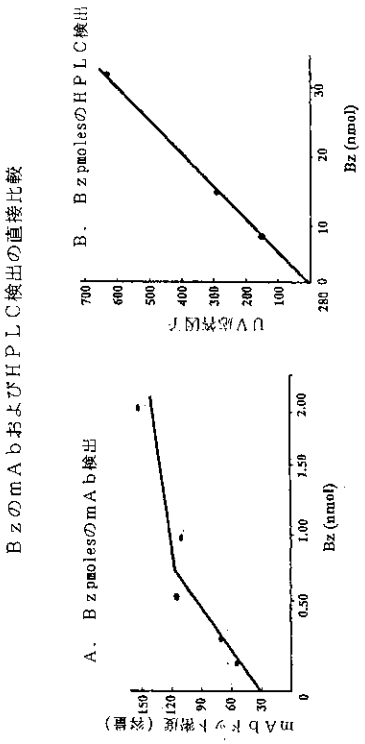


Fig. 7.

【図８】

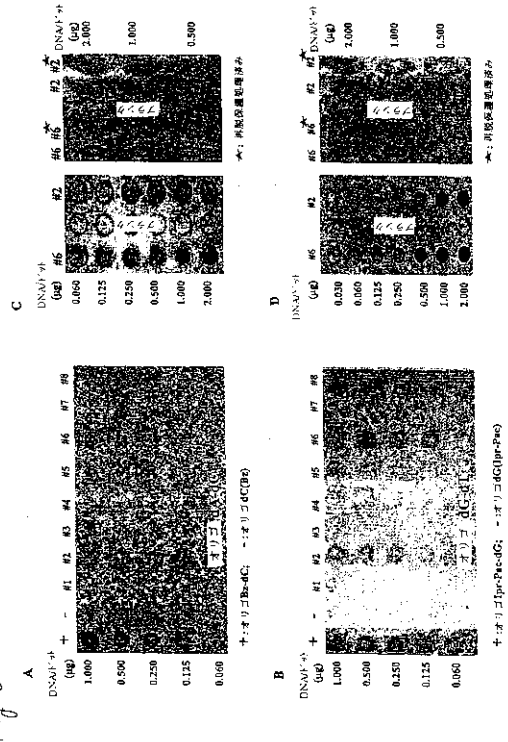


Fig. 8

【図９】

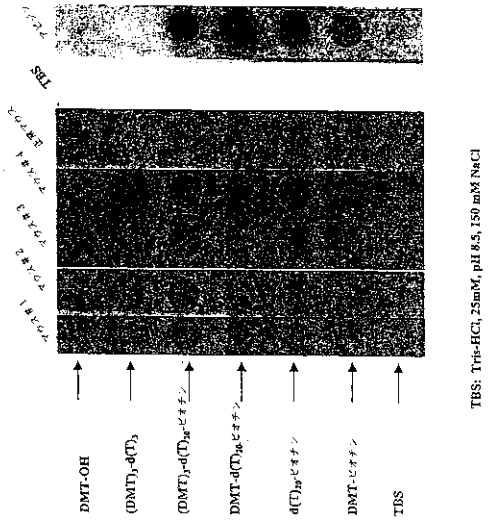


Fig. 9

【図１０】

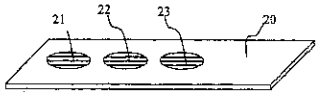


Figure 10

【図１１】

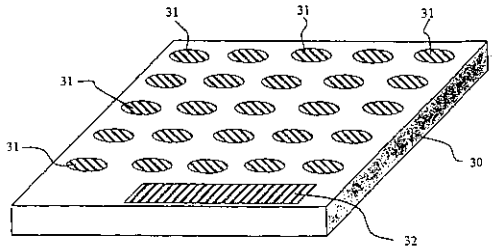


Figure 11

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(54) Title: METHODS AND COMPOSITIONS FOR DETERMINING THE PURITY OF CHEMICALLY SYNTHESIZED NUCLEIC ACIDS

(57) Abstract: This application describes an antibody that specifically binds to a synthetic oligomer (e.g., an oligonucleotide or oligopeptide) having a organic protecting group covalently bound thereto, which antibody does not bind to that synthetic oligomer when the organic protecting group is not covalently bound thereto. Methods of making and using such antibodies are also disclosed, along with cells for making such antibodies and articles carrying immobilized oligomers that can be used in assay procedures with such antibodies.

METHODS AND COMPOSITIONS FOR DETERMINING THE PURITY OF CHEMICALLY SYNTHESIZED NUCLEIC ACIDS

Paul F. Agris, Christopher D. J. Pearce, and Lloyd G. Mitchell

Related Applications

This application is a continuation-in-part of commonly owned, copending application Serial No. 09/476,975, filed December 31, 1999, the disclosure of which is incorporated by reference herein in its entirety.

Field of the Invention

The present invention concerns the detection, identification and quantification of protecting groups remaining after chemical synthesis of oligomers, particularly oligonucleotides.

Background of the Invention

Over the past decade automated chemical synthesis of nucleic acids such as DNA and RNA on solid supports has been developed. These chemical processes include the use of agents to protect the exocyclic amines of the nucleotide bases adenine, thymine, cytosine and guanine and to direct the synthesis by blocking the 2'OH of RNA's ribose. The bases within the nucleic acid product of the synthesis are deprotected upon cleavage of the nucleic acid from the solid support. However, the extent of base deprotection is not easily determined.

For example, after base deprotection of synthetic RNA, products still contain the 2'-dimethylsilyl *tert*-butyl group as a protection of the 2'OH of the ribose moiety. This protecting group is removed carefully by chemical means so as not to effect the chemistry and structure of the RNA. However, the extent of deprotection of the 2'OH is not readily determined. The nucleic acid is purified by high pressure liquid chromatography or by gel electrophoresis. However, some of the unwanted products

of the synthesis are complete nucleic acid sequences that still contain one or more protecting groups, and shorter than full length (aborted) sequences difficult to separate from full length sequences, especially for oligomers of longer than 50 nucleosides. At present, there is no easy method to determine how much of each protecting group, if any, still remains on the product, and what proportion of the product is full-length. *See generally* Davis, G.E., Gehrke, C.W., Kuo, K.C., and Agris, P.F. (1979) Major and Modified Nucleosides in tRNA Hydrolysates by High Performance Liquid Chromatography. *J. Chromatogr.* 173:281-298; Agris, P.F., Tompson, J.G., Gehrke, C.W., Kuo, K.C., and Rice, R.H. (1980) High-Performance Liquid Chromatography and Mass Spectrometry of Transfer RNA Bases for Isotopic Abundance. *J. Chromatogr.* 194:205-212; Gehrke, C.W., Kuo, K.C., McCune, R.A., Gerhardt, K.O., and Agris, P.F. (1981) Quantitative Enzymatic Hydrolysis of tRNAs: RP-HPLC of tRNA Nucleosides. *J. Chromatogr.* 230:297-308; Chromatography and Modification of Nucleosides Volumes A, B and C (Gehrke, C.W. and Kuo, K.C.T., eds.), Elsevier Publishing Co. 1990; Agris, P.F. and Sierzputowska-Gracz, H. (1990) Three Dimensional Dynamic Structure of tRNA's by Nuclear Magnetic Resonance. In Chromatography and Modification of Nucleosides (Gehrke, C.W. and Kuo, K.C.T., eds.), Elsevier Publishing Co., pp. 225-253; Agris, P.F., Hayden, J., Sierzputowska-Gracz, H., Ditson, S., Degres, J.A., Tempesta, M., Kuo, K.C. and Gehrke, C.W. (1990) Compendium on Biological, Biochemical, Chemical, Physical and Spectroscopic Properties of RNA and DNA Nucleosides. In Chromatography and Modification of Nucleosides, Elsevier Publishing Co.

The incomplete removal of the protecting group and lack of a simple assay is a problem for two industries and for numerous researchers world wide: *(i)* the multitude of companies now providing nucleic acid sequence synthesis products by overnight delivery have difficulty telling their customers the extent to which the product is deprotected; *(ii)* pharmaceutical companies cannot easily verify for regulatory agencies the purity and/or length of the therapeutic or diagnostic oligonucleotide products they seek to introduce or market. Accordingly, there is a need for simple and reliable techniques for determining the purity and proportion of full length of oligonucleotide products.

Summary of the Invention

A first aspect of the present invention is an antibody (*e.g.*, a monoclonal or polyclonal antibody) that specifically binds to a synthetic oligomer (*i.e.*, an

oligonucleotide or oligopeptide) having a organic protecting group covalently bound thereto, which antibody does not bind to that synthetic oligomer when the organic protecting group is not covalently bound thereto.

A second aspect of the present invention comprises a cell or cells, including cell cultures and isolated cells, that express an antibody as described above. Such cells include hybridoma cells, as well as recombinant cells that contain and express a heterologous nucleic acid encoding the antibody.

A third aspect of the present invention is a method for detecting incomplete deprotection of a synthetic oligomer by immunoassay, said immunoassay comprising the steps of: *(a)* contacting a synthetic oligomer to an antibody as described above, and then *(b)* detecting the presence or absence of binding of said antibody to said oligomer, the presence of binding indicating incomplete deprotection of said synthetic oligomer. Any suitable assay format can be employed, including heterogeneous and homogeneous immunoassays. For example, the immunoassay may be an immunoblot-dot assay, or may be a sandwich assay.

A fourth aspect of the present invention is a method for separating protected (including partially and completely protected) synthetic oligomers from fully deprotected synthetic oligomers. The method comprises *(a)* contacting a mixture of protected from fully deprotected synthetic oligomers to antibodies as described above, wherein the protected synthetic oligomers have the organic protecting group covalently bound thereto, so that the protected synthetic oligomers bind to the antibody; and then separating the antibodies from the fully deprotected oligomers. The antibody may be immobilized on a solid support to facilitate separation. The protected synthetic oligomer may be a partially protected synthetic oligomer (for which one application is the identification and/or purification of full-length versus aborted sequence oligomers) or a fully protected synthetic oligomer that has not undergone deprotection. Any separation format may be used, including but not limited to affinity chromatography.

A fifth aspect of the invention is an article useful for the determining incomplete deprotection of a synthetic oligomer in an immunoassay, said article comprising: *(a)* a solid support (*e.g.*, a nitrocellulose strip) having a surface portion, said surface portion having at least two separate discrete regions formed thereon; *(b)* a

first oligomer bound to one of said separate discrete regions, said first oligomer having a protecting group bound thereto; and *(c)* a second oligomer bound to another of said separate discrete regions, said second oligomer not having said protecting group bound thereto; wherein the nucleotide sequence of said first and second oligomers are the same. In a preferred embodiment, the article further comprises *(d)*a third oligomer bound to another of said separate discrete regions; said third oligomer also having said protecting group bound to said first oligomer bound thereto; wherein said third oligomer is partially deprotected; and wherein the nucleotide sequence of said first, second, and third oligomers are the same.

A sixth aspect of the present invention is a method of making an antibody that specifically binds to a synthetic oligomer having a organic protecting group covalently bound thereto, which antibody does not bind to the said synthetic oligomer when said organic protecting group is not covalently bound thereto, said method comprising the steps of: *(a)* synthesizing said synthetic oligomer on a solid particulate support (and preferably covalently bound thereto, *e.g.*, with a succinyl linker) with said organic protecting group covalently bound to said synthetic oligomer (or synthesizing a monomer of a single nucleotide on the solid support, with the single nucleotide having said protecting group covalently bound thereto); and then, without removing said oligomer from said solid support; *(b)* immunizing an animal with said synthetic oligomer bound to said solid support (or monomer bound to said solid support) in an amount sufficient to produce said antibody. Optionally, the solid support can be replaced with a carrier group such as a protein (e.g., bovine serum albumin).

In summary, the antibodies and methods of the present invention are useful in immunoassays, such as for the qualitative and quantitative detection of protecting groups used in organic synthetic processes, with particular application to oligonucleotides or peptides in research, therapeutics, diagnostics and biomedical science. The antibodies of the invention can be used in purification techniques, such as for the separation of final products from by-product contaminants. The instant invention can be used in the course of quality control of oligonucleotide and peptide synthesis, such as in the quality control of drugs for gene therapy, antisense, antigene and control of gene expression, in the quality control of biomedical polymers that may

contain protecting groups, and as probes for purification and characterization of synthetic oligomers, particularly oligonucleotides or peptides.

The present invention is explained in greater detail in the drawings herein and the specification set forth below.

Brief Description of the Drawings

Figure 1 is a dot-blot immunoassay of monoclonal antibody 1 H11, which selectively binds to oligoIbu-dG20mers.

Figure 2 is a dot-blot immunoassay of monoclonal antibody 7H3, which selectively binds to oligoBz-dC20mers.

Figure 3 shows ELISA **(A)** and dot-blot **(B)** results demonstrating specificity and detection sensitivity of a monoclonal antibody (mAb) of the commonly used protecting group, benzoyl (Bz), for the chemical synthesis of nucleic acids. Partially deprotected oligomer oligo Bz-dC (center column) can be re-treated to remove the remaining protecting groups, and re-tested with mAb **(C)**. An RNA standard with protecting groups Bz, ibu and ipr-Pac was synthesized and assayed for identification of the protecting groups with the mAb against Bz **(D)**.

Figure 4 shows ELISA **(A)** and dot-blot **(B)** results demonstrating specificity and sensitivity of a monoclonal antibody (mAb) and its detection of the commonly used protecting group, isobutryl (ibu), for the chemical synthesis of nucleic acids. Dot-blot assay with high amounts of DNA demonstrates that the ibu protecting group was recognized by the mAb no matter which nucleobase was protected **(C)**. Partially deprotected oligomer oligo Bz-dC (center column) can be re-treated to remove the remaining protecting groups, and re-tested with mAb **(D)**. An RNA standard with protecting groups Bz, ibu and ipr-Pac was synthesized and assayed for identification of the protecting groups with the mAb against ibu **(E)**.

Figure 5 shows ELISA **(A)** and dot-blot **(B)** results demonstrating specificity and sensitivity of a monoclonal antibody (mAb) and its detection of the commonly used protecting group, isopropylphenoxyacetyl (ipr-Pac), for the chemical synthesis of nucleic acids. Partially deprotected oligomers oligo ibr-Pac-dG and oligo ibu-dG (columns second from left and forth from left, respectively) can be re-treated to remove the remaining protecting groups, and re-tested with mAb **(C)**. An RNA

standard with protecting groups Bz, ibu and ipr-Pac was synthesized and assayed for identification of the protecting groups with the mAb against ipr-Pac (**D**).

Figure 6 shows a mAb dot-blot assay of protecting groups demonstrating the sensitivity and quantifiable response of the technology as related to HPLC. Dot-blot detection of Bz groups remaining on a standardized 20mer oligo dC molecule was analzyed (**A**) and a quantitation of the mAb response (**B**) was determined. The mAb response was analyzed with an increase in the amount of DNA on the dot-blot membrane (**C**). The column on the left is just the protected Bz-dC 20mer. The column on the right is the protected Bz-dC together with a 2500-fold excess of the completely deprotected oligo dC(Bz).

Figure 7 shows a direct comparison of the mAb and HPLC detection of Bz in the pmole (**A**) and nmol range (**B**), respectively.

Figure 8 shows a blind study demonstrating the detection of remaining protecting groups in commercial samples. dA-dC oligos were analyzed with anti-Bz mAb (**A**) and dG-dT oligos were analyzed with anti-ipr-Pac mAb (**B**). The oligo dA-dC samples from companies #2 and #6 were tested in higher amounts to confirm the presence of the Bz protecting group (**C**). In addition, the samples were treated to remove the remaining protecting groups using a standard protocol. The oligo dG-dT samples were assayed for the ipr-Pac protecting groups (**D**). The samples were re-treated to remove remaining protecting groups and re-analyzed as in (C).

Figure 9 shows the production and analyses of polyclonal antibody against the 5'terminal protecting group, dimethyltrityl (DMT).

Figure 10 shows a substrate carrying different oligonucleotides of the same sequence, but with varying degrees of deprotection, that may be used as a testing standard to screen similar oligonucleotides of the same sequence for varying degrees of protection or deprotection.

Figure 11 illustrates an oligonucleotide array that may be screened for the presence of protecting groups or insufficient elongation with antibodies of the present invention.

Detailed Description of the Preferred Embodiments

1. General Definitions.

"Antibody" as used herein refers to both monoclonal and polyclonal antibodies, refers to antibodies of any immunoglobulin type (including but not limited to IgG and IgM antibodies), and including antibody fragments that retain the hypervariable or binding regions thereof. Antibodies may be of any species of origin, but are typically mammalian (*e.g.*, horse, rat, mouse, rabbit, goat). Antibodies may be bound to or immobilized on solid supports such as nitrocellulose, agarose, glass, organic polymers ("plastics") and the like in accordance with known techniques, and may be labeled with or joined to other detectable groups in accordance with known techniques.

"Binding" as used herein with respect to the selective binding of an antibody to an oligomer has its usual meaning in the art. In general, to obtain useful discrimination in an immunoassay or an affinity purification technique, the antibody should bind to the protected oligomer at an affinity of at least about $k_d = 10^{-6}$, 10^{-7}, or 10^{-8} M, and should bind to the unprotected oligomer at an affinity of not greater than about $k_d = 10^{-2}$, 10^{-3}, or 10^{-4} M.

"Oligomer" as used herein refers to synthetic oligonucleotides and synthetic oligopeptides, including synthetic oligomers in the naturally occurring form such as DNA and RNA, and modified backbone chemistries as discussed below. Oligonucleotides are currently preferred in carrying out the present invention, and the instant invention is primarily explained with reference to oligonucleotides herein. However, the methods and techniques described herein may also be applied to oligopeptides, oligosaccharides, etc. (*i.e.*, any synthetically produced polymer requiring protecting groups for synthesis).

"Nucleotide" as used herein refers to a subunit of an oligonucleotide comprising a pentose, a nitrogenous heterocyclic base (typically bound to the 1 position of the pentose), and a phosphate or phosphoric acid group (typically bound at the 5' position of the pentose) but absent, or considered bound at the 3' position, in the 5' terminal nucleotide of an oligonucleotide. These structures are well known. *See, e.g.*, A. Lehninger, *Biochemistry*, 309-320). "Nucleoside" typically refers to a nucleotide, absent a phosphoric acid or phosphate group.

"Protecting group" as used herein has its conventional meaning in the art and refers to a chemical moiety, group or substituent that is coupled, typically covalently

coupled, to an atom in a molecule prior to a chemical reaction involving that molecule (typically in an organic synthesis), so that the chemical reaction is averted at the atom to which the protecting group is coupled. Typically, the protecting group is then chemically removed from the intermediate molecule for preparation of the final product, although removal techniques may not be entirely successful leading to only partial deprotection of the final product (i.e., the presence of at least one protecting group remaining on that molecule). Protecting groups may be intentionally left on a molecule for purposes of generating or testing an antibody as described herein.

"Deprotection" or "deprotected" as used herein refers to the absence of protecting groups employed during chemical oligonucleotide synthesis from a molecule. Such protecting groups are described below. The presence of such a protecting group may indicate insufficient elongation of the oligonucleotide, when the protecting group is chain terminating. Chemically synthesized oligonucleotides are ideally fully deprotected, but the present invention is employed to detect partial or incomplete deprotection of such oligonucleotides (that is, the presence of at least one protecting group as described below in the oligonucleotide).

"Base" as used herein with respect to oligonucleotides refers to a nitrogenous heterocyclic base which is a derivative of either purine (*e.g.,* adenine, guanine) or pyrimidine (*e.g.,* uracil, thymine, cytosine). Pyrimidine bases are bound to the pentose by the 1 ring nitrogen; Purine bases are bonded to the pentose by the 9 ring nitrogen. Preferred bases are those that contain a free amino group, such as guanine, adenine, and cytosine (the protecting group is then covalently bound to the free amino group by substitution of one, or both, of the hydrogens on the free amino group). However, the present invention may be used with any purine or pyrimidine base, whether standard or modified/rare, that contains a free amino group for protection, or other group requiring protection during synthesis thereof in an oligonucleotide. Examples of standard and modified/rare bases are those found in the nucleosides set forth in **Table 1** below.

Table 1. Standard and modified nucleosides and their standard abbreviations.

abbreviation	base
U	uridine
C	cytidine
A	adenosine
G	guanosine
T	thymidine
?A	unknown modified adenosine
m1A	1-methyladenosine
m2A	2-methyladenosine
i6A	N^6–isopentenyladenosine
ms2i6A	2-methylthio-N^6-isopentenyladenosine
m6A	N^6-methyladenosine
t6A	N^6-threonylcarbamoyladenosine
m6t6A	N^6-methyl-N^6-threonylcarbomoyladenosine
ms2t6A	2-methylthio-N^6-threonylcarbamoyladenosine
Am	2′-O-methyladenosine
I	Inosine
m1I	1-methylinosine
Ar(p)	2′-O-(5-phospho)ribosyladenosine
io6A	N^6-(cis-hydroxyisopentenyl)adenosine
?C	Unknown modified cytidine
s2C	2-thiocytidine
Cm	2′-O-methylcytidine
ac4C	N^4-acetylcytidine
m5C	5-methylcytidine
m3C	3-methylcytidine
k2C	lysidine
f5C	5-formylcytidine
f5Cm	2′-O-methyl-5-formylcytidine
?G	unknown modified guanosine
Gr(p)	2′-O-(5-phospho)ribosylguanosine
m1G	1-methylguanosine
m2G	N^2-methylguanosine
Gm	2′-O-methylguanosine
m22G	N^2N^2-dimethylguanosine
m22Gm	N^2,N^2,2′-O-trimethylguanosine
m7G	7-methylguanosine
fa7d7G	archaeosine
Q	queuosine
manQ	mannosyl-queuosine
galQ	galactosyl-queuosine
Yw	wybutosine
o2yW	peroxywybutosine
?U	unknown modified uridine

abbreviation	base
mnm5U	5-methylaminomethyluridine
s2U	2-thiouridine
Um	2'-O-methyluridine
s4U	4-thiouridine
ncm5U	5-carbamoylmethyluridine
mcm5U	5-methoxycarbonylmethyluridine
mnm5s2U	5-methylaminomethyl-2-thiouridine
mcm5s2U	5-methoxycarbonylmethyl-2-thiouridine
cmo5U	uridine 5-oxyacetic acid
mo5U	5-methoxyuridine
cmnm5U	5-carboxymethylaminomethyluridine
cmnm5s2U	5-carboxymethylaminomethyl-2-thiouridine
acp3U	3-(3-amino-3-carboxypropyl)uridine
mchm5U	5-(carboxyhydroxymethyl)uridinemethyl ester
cmnm5Um	5-carboxymethylaminomethyl-2'-O-methyluridine
ncm5Um	5-carbamoylmethyl-2'-O-methyluridine
D	Dihydrouridine
ψ	pseudouridine
m1ψ	1-methylpseudouridine
ψm	2'-O-methylpseudouridine
m5U	ribosylthymine
m5s2U	5-methyl-2-thiouridine
m5Um	5,2'-O-dimethyluridine

See Sprinzl et al., *Nucleic Acids Res.* **26**, 148 (1998).

Applicants specifically intend that the disclosures of all United States patent references cited herein be incorporated by reference herein in their entirety.

2. Protecting groups.

The particular protecting group will depend upon the oligomer being synthesized and the methodology by which that oligomer is synthesized.

For the synthesis of oligonucleotides, suitable protecting groups include alkyl, aryl, alkylaryl, arylalkyl groups, which may contain one or more hetero atoms such as N, O, or S, and which may be substituted or unsubstituted (*e.g.*, a carbonyl group). Examples of protecting groups include, but are not limited to, the following: acetyl; isobutyryl; 2-(t-butyldiphenyl-silyloxymethyl)benzoyl; naphthaloyl; iso-butyryloxycarbonyl; levulinyl; fluorenylmethoxycarbonyl; 2-nitrothiophenyl; 2,2,2-trichloro-t-butoxycarbonyl, ethoxycarbonyl; benzyloxycarbonyl; p-nitrophenyl-

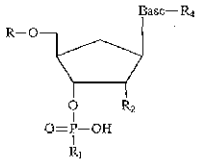
ethyloxycarbonyl; N'N-dimethylformamidine; formyl; benzoyl, toluyl; 2,4-6-trimethylbenzoyl; anisoyl; 2,4-dimethylphenyl; 2,4,6-trimethylphenyl; triphenylthiomethyl; pivoloiloxymethyl; t-butoxycarbonyl; p-nitrophenylethyl; methoxyethoxymethyl; butylthiocarbonyl; 2-methyl-pyridine-5-yl; 2-nitrothiophenyl; 2,4-dinitrothiophenyl; 2-nitro-4-methylthiophenyl; p-nitrophenylsulphonylethyl; 5-chloro-8-hydroxyquinoline; thiophenyl; β-cyanoethyl; phenylethyl; p-nitrophenylethyl; pyridylethyl; 2-N-methylimidazolylphenyl; methyl; allyl; trichloroethyl; dibenzoyl; p-nitrophenylethoxycarbonyl; benzoyl and substituted derivatives thereof; 2(acetoxymethyl) benzoyl; 4,4',4"-tris-(benzyloxy)trityl; 5-methylpyridyno-2-yl; phenylthioethyl; dipehylcarbamoyl; 3,4-dimethoxybenzyl; 3-chlorophenyl; 2-nitrophenyl; 9-pnenylxanthen-9-yl; 9-(p-methoxyphenyl)xanthen-9-yl; 9-(p-ocatadecyloxyphenyl)xanthen-9-yl; "bridged" bis-dimethoxytrityl groups; phthaloyl; succinyl; benzensulphonylethoxycarbonyl; 4,4',4"-tris(bevulinyloxy)trityl; p-phenylazophenyloxycarbonyl; o-substituted benzoyl, 4,4'4"-tris-(4,5-dichlorophalimidin)trityl; levelinyl; alkyloxy and aryloxyacetyl; 1,3-benzodithiol-2-yl; tetrahydrofuranyl; [2-(methylthio)phenyl]thiomethyl; 1-(2-chloroethyoxy)ethyl; 1-[(2-fluoro-phenyl]4-methoxy piperidin-4-yl; 4-methoxytetrahydropyran-4-yl; (1-methyl-1-methoxy)ethyl; tetrahydropyranyl; 3-methoxy-1,5-dicarbomethoxypentam-3-yl; 2-nitrobenzyl; benzyl; 4-nitrophenylethyl-sulphonyl; t-butyldimethylsilyl; 4-methoxybenzyl; 3,4-dimethoxybenzyl; 9-p-methoxyphenylthioxanthen-9-yl; compounds of the formula $R_1R_2R_3C$-, wherein R_1, R_2, and R_3 are each independently selected from the group consisting of phenyl, p-monomethoxyphenyl, o-monomethoxphenyl, biphenyl, p-fluoropnehyl, p-chlorophenyl, p-methylphenyl, p-nitrophenyl, etc.

3. *Oligonucleotides.*

Synthetic oligonucleotides that contain protecting groups and may be used to carry out the present invention include both the naturally occurring forms such as DNA and RNA, and those with modified backbone chemistries, such as poly (phosphate derivatives) such as phosphonates, phosphoramides, phosphonamides, phosphites, phosphinamides, *etc.*, poly (sulfur derivatives) *e.g.*, sulfones, sulfonates, sulfites, sulfonamides, sulfenamides, *etc.* It will be noted that antibodies of the

invention may be characterized by their selective binding to particular "reagent" or "benchmark" oligonucleotides, but the same antibodies may also bind to a variety of other oligonucleotides (e.g., longer nucleotides) or other compounds that contain the same protecting group.

For example, an oligonucleotide to which the antibody selectively binds may consist of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I) below:



(I)

wherein:

R is H or a protecting group, such as dimethoxytrityl; subject to the proviso that R is a covalent bond to an adjacent nucleotide when said protected base is not a 5' terminal nucleotide in said oligonucleotide;

R_1 is H or a protecting group such as β-cyanoethyl; subject to the proviso that R_1 is a covalent bond to an adjacent nucleotide when said protected base is not a 3' terminal nucleotide in said oligonucleotide;

R_2 is H or $-OR_3$;

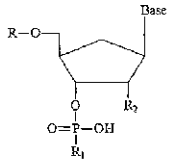
R_3 is H or a protecting group such as *tert*-butyldimethylsilyl;

Base is a purine or pyrimidine base; and

R_4 is a protecting group bonded to an amino group of said base, such as a protecting group is selected from the group consisting of acetyl (Ac), benzoyl (Bz), dimethylformamidine (dmf), isobutyrl (Ibu), phenoxyacetyl (Pac), and isopropyl-phenoxyacetyl (Ipr-pac);

and further subject to the proviso that when one of R, R_1, R_2 and R_4 is a protecting group, then the others of R, R_1, R_3 and R_4 are not protecting groups.

In one particular embodiment of the foregoing, the antibody may be one that selectively binds to an oligonucleotide that consists of from 3 to 20 nucleotides and has a 5' nucleotide, and wherein said 5' nucleotide is a protected nucleotide according to Formula (I):



(I)

wherein:

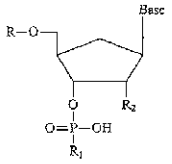
R is a protecting group such as dimethoxytrityl;

R_1 is a covalent bond to an adjacent nucleotide;

R_2 is -H or –OH; and

Base is a purine or pyrimidine base.

In another particular embodiment of the foregoing, the antibody may be one that selectively binds to an oligonucleotide that consists of from 3 to 20 nucleotides and has a 3' nucleotide, and wherein said 3' nucleotide is a protected nucleotide according to Formula (I):



(I)

wherein:

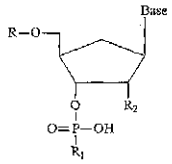
R is a covalent bond to an adjacent nucleotide;

R_1 is a protecting group such as β-cyanoethyl;

R_2 is H or –OH; and

Base is a purine or pyrimidine base.

In another particular embodiment of the foregoing, the antibody may be one that selectively binds to an oligonucleotide that consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I):



(I)

wherein:

R is a covalent bond to an adjacent nucleotide;

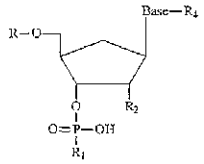
R_1 is a covalent bond to an adjacent nucleotide;

R_2 is $-OR_3$;

R_3 a protecting group such as *tert*-butyldimethylsilyl; and

Base is a purine or pyrimidine base.

In still another particular embodiment of the foregoing, the antibody may be one that selectively binds to an oligonucleotide that consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I):



(I)

wherein:

R is a covalent bond to an adjacent nucleotide;

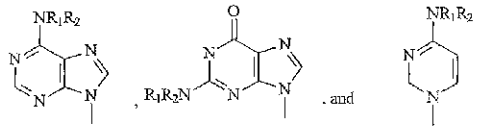
R_1 is a covalent bond to an adjacent nucleotide;

R_2 H or –OH;

Base is a purine or pyrimidine base; and

R_4 is a protecting group bonded to an amino group of said base, such as acetyl, benzoyl, dimethylformamidine, isobutyryl, phenoxyacetyl, and isopropyl-phenoxyacetyl.

Thus, examples of protected bases that may be employed in the structures shown above include, but are not limited to, adenine, guanine, and cytosine, as follows:



wherein R_1 and R_2 are both H in an unprotected base, and either R_1 or R_2 are a protecting group as described above (*e.g. Pac, Ipr-pac, Ibu, Bz, Ac, dmf*) for a protected base. Likewise, modified nucleosides have protecting groups at the modifications that are chemically reactive.

In one embodiment of the invention, the oligonucleotides are peptide nucleic acids, and the protecting groups are those protecting groups employed in the synthesis of peptide nucleic acids, including but not limited to those described in U.S. Patent No. 6,133,444.

In still another particular embodiment of the foregoing, the antibody may be one that selectively binds to an oligonucleotide that consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected with a photolabile protecting group, including but not limited to those described in U.S. Patents Nos. 5,744,101 and 5,489,678 (assigned to Affymax).

4. Antibodies.

As noted above, the present invention provides antibodies (*e.g.*, a monoclonal or polyclonal antibody) that specifically bind to a synthetic oligonucleotide having a organic protecting group covalently bound thereto, which antibody does not bind to said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto.

The antibody may be provided immobilized on (or bound to) a solid support in accordance with known techniques, or may be provided in a free, unbound form (*e.g.*, lyophilized, frozen, in an aqueous carrier, etc.). Whether or not an antibody is immobilized will depend upon the particular immunoassay or affinity purification technique in which the antibody is used, and is determined by the known parameters for such techniques. Similarly, the antibody may be bound to or conjugated with suitable detectable groups, such as an enzyme (*e.g.*, horseradish peroxidase), a member of a binding pair such as biotin or avidin, a radioactive group or a fluorescent group such as green fluorescent protein, also in accordance with known techniques, typically depending upon the immunoassay format in which the antibody is used.

5. Immunoassay Methods.

The present invention provides a method for detecting incomplete deprotection of a synthetic oligonucleotide (including aborted sequences that still contain a protecting group) by immunoassay. In general, such an immunoassay comprises the steps of: *(a)* contacting a synthetic oligonucleotide to an antibody as described above, and then *(b)* detecting the presence or absence of binding of said antibody to said oligonucleotide, the presence of binding indicating incomplete deprotection of said synthetic oligonucleotide. Any suitable assay format can be employed, including heterogeneous and homogeneous immunoassays. For example, the immunoassay may be an immunoblot-dot assay, or may be a sandwich assay. The oligonucleotides being tested for deprotection may be in any suitable form, such as in solution or immobilized on a solid support.

In a preferred embodiment, the detection method employs a "dip stick" or the like, in which binding of the antibody to the test oligonucleotide is compared to binding of the antibody to a set of known oligonucleotides, all immobilized on a common solid support. Such an article, as illustrated in **Figure 10**, useful for

determining incomplete deprotection of a synthetic oligonucleotide in an immunoassay, comprises: *(a)* a solid support (*e.g.*, a nitrocellulose strip) **25** having a surface portion, said surface portion having at least two separate discrete regions **26, 27** formed thereon; *(b)* a first oligonucleotide bound to one of said separate discrete regions, said first oligonucleotide having a protecting group bound thereto (*e.g.*, at least one protecting group); and *(c)* a second oligonucleotide bound to another of said separate discrete regions, said second oligonucleotide not having said protecting group bound thereto; wherein the nucleotide sequence of said first and second oligonucleotides are the same. In a preferred embodiment, the article further comprises *(d)* a third oligonucleotide bound to another of said separate discrete regions **28**; said third oligonucleotide also having said protecting group bound to said first oligonucleotide bound thereto; wherein said third oligonucleotide is partially deprotected (*i.e.*, has a number of protecting groups covalently bound thereto which is intermediate between that bound to the first and second oligonucleotide, e.g., at least one, two three or four more protecting groups than the first oligonucleotide, up to at least 10, 20 or more protecting groups than the first oligonucleotide); and wherein the nucleotide sequence of said first, second, and third oligonucleotides are the same. Of course, still more oligonucleotides carrying varying numbers of protecting groups may be included on the substrate in additional separate and discrete locations, if desired. The discrete regions to which the separate oligonucleotides are bound may be in any form, such as dots.

6. Affinity Purification Methods.

In addition to immunoassays, the present invention also provides affinity purification techniques for the separation of fully deprotected oligonucleotides from partially deprotected (including fully protected) oligonucleotides (*e.g.*, both oligonucleotides that have been subjected to a deprotection process to remove the protecting group, and oligonucleotides that have not). Such a procedure typically comprises *(a)* contacting a mixture of protected and fully deprotected synthetic oligonucleotides to antibodies as described above, wherein the protected synthetic oligonucleotides have the organic protecting group for which the antibody is selective covalently bound thereto, so that the protected synthetic oligonucleotides bind to the

antibody, and then separating said antibodies from said fully deprotected oligonucleotides. The antibody may be immobilized on a solid support to facilitate separation. The protected synthetic oligonucleotide may be a partially protected synthetic oligonucleotide, or a fully protected synthetic oligonucleotide that has not undergone deprotection. Any separation format may be used, including but not limited to affinity chromatography.

7. Production of Antibodies.

A method of making an antibody that specifically binds to a synthetic oligonucleotide having a organic protecting group covalently bound thereto, which antibody does not bind to the said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto, comprises the steps of: *(a)* synthesizing the synthetic oligonucleotide on a solid particulate support (and preferably covalently bound thereto, *e.g.*, with a succinyl linker) with the organic protecting group covalently bound to said synthetic oligonucleotide; and then, without removing the oligonucleotide from said solid support; and *(b)* immunizing an animal with the synthetic oligonucleotide bound to the solid support in an amount sufficient to produce the antibody. In addition, a single nucleotide can be bound to the solid particulare support with the organic protecting group bound thereto, and used as described hereinabove.

The synthesis step may be carried out on the solid support in accordance with known techniques. The solid support may be in particulate form prior to synthesis, or may be fragmented into particles after synthesis. In general, the solid supports are beads, which may be completely solid throughout, porous, deformable or hard. The beads will generally be at least 10, 20 or 50 to 250, 500, or 2000 μm in diameter, and are most typically 50 to 250 μm in diameter. Any convenient composition can be used for the solid support, including cellulose, pore-glass, silica gel, polystyrene beads such as polystyrene beads cross-linked with divinylbenzene, grafted copolymer beads such as polyethyleneglycol/polystyrene, polyacrylamide beads, latex beads, dimethylacrylamide beads, composites such as glass particles coated with a hydrophobic polymer such as cross-linked polystyrene or a fluorinated ethylene polymer to which is grafted linear polystyrene, and the like. Where separate discrete

solid supports such as particles or beads are employed, they generally comprise from about 1 to 99 percent by weight of the total reaction mixture.

In a preferred embodiment, the synthesizing step is followed by the step of fragmenting the solid support (*e.g.*, by crushing) prior to the immunizing step. Polyclonal antibodies may be collected from the serum of the animal in accordance with known techniques, or spleen cells may be collected from the animal, a plurality of hybridoma cell lines produced from the spleen cells; and then a particular hybridoma cell line that produces the antibody isolated from the plurality of hybridoma cell lines.

A particular protocol for the production of antiserum/polyclonal antibodies and monoclonal antibodies against protecting groups used in nucleic acid and other synthesis typically involves the following steps: *(a)* preparation of oligonucleotides and others that contain or do not contain protecting groups; *(b)* immunization of animals with those preparations; *(c)* screening of animals to identify those that exhibit antibodies against protecting groups; *(d)* production of monoclonal antibody by classical fusion method; *(e)* optionally, production of scFab, Fab fragments and whole antibody molecules by antibody engineering; and *(f)* evaluation and characterization of monoclonal antibodies against the protecting groups. Each of these steps is discussed in greater detail below.

Synthetic oligonucleotides that contain protecting groups can be synthesized in a variety of ways known to those skilled in the art. For example, protecting groups can be attached to individual nucleotides that are linked to controlled pore glass (CPG) beads. An example is:

CPG bead---dT (only with DMT group).

In the alternative, protecting groups may be attached to oligonucleotide chains that are linked to CPG beads. Examples include:

Pac-dA---Pac-dA---CPG beads with Bz-dC and Ibu-dG;

Ipr-Pac-dG---Ipr-Pac-dG--- CPG beads with Bz-dC and Ibu-dG;

Ac-dC---Ac-dC--- CPG beads with Bz-dC and Ibu-dG;

dmf-G---dmf-G--- CPG beads with Bz-dC and Ibu-dG; and

mixtures of the four oligonucleotides described above.

In another alternative, protecting groups may be attached to oligonucleotide chains that are partially deprotected (the procedure for deprotection will be described bellow). Examples include:

Poly dT20mers (only with DMT group);

Poly dT20mers (only with cyanoethyl groups);

Poly Ibu-dG 20mers (partially deprotected);

Poly Ipr-Pac-dG 20mers (partially deprotected);

Poly Bz-dC 20mers (partially deprotected);

Poly Pac-dA 20mers (partially deprotected); and

Poly Ac-dC 20mers (partially deprotected).

Synthetic oligonucleotides prepared as described herein may be partially deprotected as follows: *(a)* add 30% ammonium hydroxide solution to synthetic polynucleotides, then incubate at room temperature for different time periods (5, 10 and 30 min); *(b)* take the ammonium solution of treated oligomers and add into 1:1 diluted acetic acid pre-cooled at 4° C and according to 1:4 ratio of ammonium to acetic acid; *(c)* keep samples in ice bath for 30 min; *(d)* dry samples with speed-Vac; *(e)* dissolve the dried pellets in water; *(f)* desalt samples with Sephadex G-25 column; *(g)* dry samples with speed-Vac; and *(h)* dissolve the desalted samples in water.

Synthetic oligonucleotides prepared as described herein may be completely deprotected by any suitable technique. One particular technique is as follows: *(a)* add 30% ammonium hydroxide solution to synthetic oligonucleotides, then incubate at 65 ° C for 6 hrs; *(b)* dry samples with speed-Vac; *(c)* dissolve the dried pellets in water; *(d)* desalt samples with Sephadex G-25 column; and *(e)* dry samples with speed-Vac; *(f)* redissolve desalted samples in water.

Partially and completely deprotected oligonucleotides may be characterized for further use or to verify procedures by any suitable means, including but not limited to gel electrophoresis, urea-acrylamide gel electrophoresis, 5'end labeling with T4 polynucleoide kinase, HPLC analysis, mass spectrometry, etc.

Suitable animals can be immunized with the oligonucleotides described above by parenteral injection of the oligonucleotide in a suitable carrier, such as sterile saline solution. Injection may be by any suitable route, including but not limited to

subcutaneous, intraperitoneal, intravenous, intraarterial, intramuscular, etc. Suitable animals are typically mammals, including mice, rabbits, rats, etc.

In a particular embodiment, for the production of monoclonal antibodies, young female BALB/c mice are used, and the time course of injection of the antigen material is:

first day	initial injection
14th day	first boosting
28th day	second boosting
4 day before fusion	final boosting

Additional injections may be employed if desired. The antigen amount may be 50 μg or 100 μg of oligonucleotides unprotected (for control antibody) or protected, for each mouse per time. When, as preferred, beads or other solid support used as the support for oligonucleotide synthesis are injected into the animal, the beads or particles are suspended in water, then injected into mice. If a nucleotide solution is used, then the solution is mixed with complete or incomplete Freund's adjuvant and injected into mice.

Polyclonal antibodies can be harvested from animals immunized or innoculated as described above in accordance with known techniques, or spleen cells harvested from the animals, hybridoma cell lines produced from the spleen cells, and the hybridoma cell lines screened for the production of desired antibodies, also in accordance with known techniques.

Oligonucleotides that contain or do not contain biotin molecules at 3' or 5' ends (for ELISA assay as described below) may be synthesized in accordance with standard techniques. Examples are:

Poly Ibu-dG 20 mers (with or without biotin);

Poly Ibu-dA 20 mers (with or without biotin);

Poly Ibu-dC 20 mers (with or without biotin);

Poly Ipr-Pac-dG 20 mers (with or without biotin);

Poly Bz-dC 20 mers (with or without biotin);

Poly Bz-dA 20 mers (with or without biotin);

Poly dT 20 mers (with or without biotin);

Poly Pac-dA 20 mers (with or without biotin);

Poly Ac-dC 20 mers (with or without biotin); and

Poly dmf-G 20 mers (with or without biotin).

Antibodies produced as described above may be characterized by any suitable technique to determine the binding properties thereof, including but not limited to Western blot and immunodot-blot.

In addition to the use of polyclonal and monoclonal antibodies, the present invention contemplates the production of antibodies by recombinant DNA, or "antibody engineering" techniques. For example, mRNA isolated from hybridoma cells may be used to construct a cDNA library and the sequence encoding whole antibody or antibody fragments (*e.g.*, scFab or Fab fragments) isolated and inserted into suitable expression vector, and the expression vector inserted into a host cell in which the isolated cDNA encoding the antibody is expressed.

Monoclonal Fab fragments may be produced in *Escherichia coli* by recombinant techniques known to those skilled in the art. See, e.g., W. Huse, *Science* 246, 1275-81 (1989).

8. Screening of Antibodies.

Screening sera and hybridoma cell culture media for protecting group specific antibodies may be carried out as follows:

A. Sera

1. Pre-immune (prior to immunization) sera are collected by standard means from the mice to be inoculated with protecting group conjugated to a solid support (directly or through an oligomer).

2. Post-inoculation sera are also collected.

3. An ELISA assay is performed in which the specific protecting group remains on a biotinylated oligonucleotide conjugated to the microtiter plate. Other microtiter plate wells contain control oligomers that have no protecting groups, or oligonucleotides with other protecting groups. The secondary antibody is a goat anti-mouse IgG with a conjugated phosphotase for visualization of antibody

4. Those mice that have positive activity against the specific protecting group are boosted and sacrificed for the production of hybridomas.

B. Hybridoma cell culture media

1. Approximately 1000 cultures are generated from each spleen hybrid cell production.

2. Cultures are grown in microtiter plate wells, 96 well plates.

3. Culture medium is removed from each well and used in ELISA assays as described above in which each of the ~1000 microtiter plate wells contain the protected oligonucleotide conjugated to the plate.

4. Those cultures producing antibody that has positive activity are transferred to larger culture wells, 24 well microtiter plates.

5. Culture media from the larger cultures are re-tested for activity against the protecting group and are also assayed for specificity; ie. controls of no protecting group and of other protecting groups.

6. Those cultures that are positive are cloned out (diluted), re-tested and cloned out again to the point that each final culture must be the result of one cell; ie. mono-culture. Media from these final cultures are thoroughly assessed for specificity and affinity. Specificity and affinity are assessed using a dot-blot assay.

C. Dot-Blot assays in lieu of ELISA assays

1. Antibodies against some protecting groups are not tractable to being tested in the microtiter plate well environment and must be tested using a dot-blot assay. One example is the 5'-terminal protecting group, dimethyl-trityl (DMT).

2. The Dot-blot assay on a nitrocellulose membrane is accomplished as described elsewhere in the application for most purposes. However, this is not possible in assessing antibody production by ~1000 microtiter well cultures with little media available. Thus, a novel adaptation has been developed.

a) The protected oligonucleotide is attached in dots to the nitrocellulose using UV-crosslinking. With DMT, the presence of the 5'-DMT on the membrane is confirmed by treatment of a dot with mild acid – the dot turns yellow-orange. The presence of the 3'-biotin can be confirmed with a commercial avidin stain.

b) The membrane is blocked (see dot-blot assay).

b) The dry membrane dots are carefully marked (pencil) and "punched" out of the membrane.

c) Individual dots are added to the cell culture media in individual micortiter plate wells and incubated.

d) The individual dots are removed and passed on through the washing, secondary antibody, phosphotase reaction and color development using microtiter plate wells with the appropriate reagents.

e) Those dots that are positive are related back to the original microtiter plate well cultures from which the small amount of culture media was obtained.

f) Further culturing and cloning is accomplished as described in B.

9. Testing of Microarrays.

The present invention may be used to test or screen oligonucleotides that are immobilized on a solid support such as a microarray for insufficient deprotection or elongation of the oligonucleotides synthesized thereon.

Solid supports used to carry out the present invention are typically discrete solid supports. Discrete solid supports may be physically separate from one another, or may be discrete regions on a surface portion of a unitary substrate. Such "chip-type" or "pin-type" solid supports are known. *See, e.g.*, U.S. Patent No. 5,143,854 to Pirrung; U.S. Patent No. 5,288,514 to Ellman (pin-based support); U.S. Patent No. 5,510,270 to Fodor et al. (chip-based support). Additional non-limiting examples of oligonucleotide arrays which may be used to carry out the present invention, and methods of making the same, include but are not limited to those described in U.S. Patents Nos. 5,631,734; 5,599,695; 5,593,839; 5,578,832; 5,510,270; 5,571,639; 6,056,926; 5,445,934; and 5,703,223. Such devices may be used as described therein to carry out the instant invention.

The solid support or substrate from which the array is formed may be comprised of any suitable material, including silicon. The oligonucleotides may be polymerized or grown in situ from monomers (or individual nucleotides) *in situ* on the microarray (in which case none of the currently available techniques for detecting protecting groups would be useful for detecting incomplete deprotection or elongation of the oligonucleotides on the array, as one cannot pass the solid support through an analytical device) or the oligonucleotides may be polymerized separately and then linked to the appropriate regions of the solid support. The array may include any number of different oligonucleotides in different separate and discrete regions

thereon, examples including arrays of at least 1,000, at least 2,000, at least 10,000, or at least 20,000 different oligonucleotides in different separate and discrete regions.

In general, a method of screening an oligonucleotide array for insufficient deprotection or insufficient elongation of oligonucleotides therein comprises the steps of:

(a) providing an oligonucleotide array as described above;

(b) providing an antibody as described above (that is, an antibody that specifically binds to a synthetic oligonucleotide having an organic protecting group covalently bound thereto, which antibody does not bind to said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto). Preferably the antibody is one that specifically binds to an oligonucleotide having a protecting group, where the protecting group was employed in the course of the organic synthesis of oligonucleotides carried by that array. Then;

(c) contacting said oligonucleotide array to said antibody to thereby detect the presence of insufficient deprotection or insufficient elongation of oligonucleotides therein. Such detection, which may be qualitative or quantitative, may be carried out by any suitable immunoassay technique as described above.

In the method, steps *(b)* to *(c)* may be repeated at least once, with a different antibody on each repetition, so that a plurality of different protecting groups which may be present on oligonucleotides in the array may be detected.

Preferably, once insufficient deprotection (the presence of protecting groups) in oligonucleotides in one or more (e.g., plurality) of the separate and discrete regions is detected, the method further comprises generating a record or indicia recording the presence of insufficient deprotection or insufficient elongation of oligonucleotides in the least one separate and discrete location (or plurality of separate and discrete locations) on the array. The indicia may be a qualitative or quantitative indicia of insufficient deprotection (including insufficient elongation).

The foregoing methods provide a correctable oligonucleotide array as illustrated in **Figure 11**. The array comprises, in combination:

(a) a substrate **30** having a plurality of different oligonucleotides immobilized thereon, with the different oligonucleotides immobilized in different separate and discrete locations **31** on said substrate; and

(b) a plurality of indicia associated with said array, these indicia recording the presence of insufficient deprotection or insufficient elongation of a plurality of different oligonucleotides, said different oligonucleotides located in separate and discrete locations on said array. These indicia may be printed in a region of the array **32** by a technique such a microlithography, printed on conventional medium such as paper and shipped with the array, stored in a memory or memory device connected to or formed on the array chip (which may be incorporated at location 32), provided in a separate data or computer file which may be provided on a computer-readable medium such as a floppy diskette or CD-ROM, stored on a web site on the world wide web for downloading by the end user of the array, etc. When the indicia are provided in a separate data file, the array preferably further includes an identifier such as a code number formed on, connected to or associated with the array (e.g., printed on a package containing the array, or on an information sheet packaged with the array, and/or printed directly on the array). The indentifer may then be associated with the separate indicia (e.g., printed on a data sheet, used as a pass-word, file identifier and/or access code for the computer file, etc.) to insure the correct indicia containing the record of insufficient deprotection and/or elongation are ultimately associated with the array by the ultimate end user of the array.

A data device or memory device connected to the array may be carried out in accordance with known techniques, as described in U.S. Patents Nos. 5,925,562; 6,017,496; 5,751,629; and 5,741,462, and such devices used as described therein to carry out the instant invention.

The end user of the array may utilize the indicia described above to compensate for insufficient deprotection or insufficient elongation of oligonucleotides on said array in a method comprising:

(a) providing a substrate as described above.

(b) providing at least one, or a plurality of, indicia associated with said array as described above.

(c) providing a test compound. The test compound may be a member of a library of test compounds, and may be any suitable compound such as a protein, peptide or oligonucleotide (e.g., a DNA or RNA, such as mRNA); and then

(d) detecting the binding of said test compound to at least one of said pluraiity of different oligonucleotides (e.g., by contacting the test compound to the array); and then

(d) detecting determining the degree of binding (including simply the presence or absence of binding) of the test compound to one or more oligonucleotides on the array from *(i)* said detected binding and *(ii)* said indicia recording the presence of insufficient deprotection or insufficient elongation. Thus, insufficient deprotection or insufficient elongation of oligonucleotides in one or more locations in the array may be compensated for during the determining step. Such compensation may be achieved by any means, including ignoring particular separate and discrete regions on the array (e.g., in favor of other separate and discrete regions of the array that contain the same oligonucleotide). In another example, if one or more locations contain insufficient deprotection or elongation such that binding to those locations is reduced, the binding data derived from an experiment with that array can be adjusted upwards for those locations to indicate greater binding than that which would otherwise be indicated without the control made possible by the recorded indicia. The detecting or determining step may be carried out by any suitable means, such as generating a color indication of degree of binding, generating a numeric indication of degree of binding, generating a graphic or other symbolic indication of degree of binding, etc. The degree of binding may be an indication of binding is binding affinity, binding amount, or both binding affinity and binding amount, but is typically an indication of the amount of test compound that binds to a particular separate and discrete region of the array.

The present invention is explained in greater detail in the following non-limiting Examples.

EXAMPLE 1

Synthesis of Oligonucleotides

Synthesis was performed on an ABI DNA/RNA Synthesizer, Model 394 (PE Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404) according to manufactories protocol. Slightly modified 1 micromolar scale cycle was used during

synthesis (see manufacturer's instructions). The primary starting materials (and suppliers/manufacturers in parentheses) were as follows:

Activator (0.45 M tetrazole in acetonitrile), CAP A (acetic anhydride/ tetrahydrofuran/ 2,6 lutidine), CAP B (N-methyl imidazole/tetrahydrofuran) and oxidizer (0.02 M iodine/pyridine/THF/H2O) (Prime Synthesis)

Pac-dA (5'-dimethoxytrityl-N-phenoxyacetyl-2'-deoxyAdenosine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research)

Ipr-Pac-dG (5'-dimethoxytrityl-N-p-isopropyl-phenoxyacetyl-2'-Guanosine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research)

Ac-dC (5'-dimethoxytrityl-acetyl-2'-deoxycytidine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research)

dmf-G (5'-dimethoxytrityl-dimethylformamidine-Guanosine,2'-O-TBDMS-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research)

Bz-dC---CPG beads (5'-dimethoxytrityl-N-benzoyl-2'-deoxycytidine, 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite-succinyl linker-beads (3000 Ang) (CPG Inc.)

Ibu-dG---CPG beads (5'-dimethoxytrityl-N-isobutyl—2'-deoxycytidine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite-succinyl linker-beads (3000 Ang) (CPG Inc.)

The following compounds were synthesized, the compounds being linked to beads as shown:

Pac-dA---Pac-dA---Bz-dC---succinyl linker---Beads

Pac-dA---Pac-dA---Ibu-dG---succinyl linker---Beads

Ipr-Pac-dG---Ipr-Pac-dG---Bz-dC---succinyl linker---Beads

Ipr-Pac-dG---Ipr-Pac-dG---Ibu-dG---succinyl linker---Beads

Ac-dC---Ac-dC---Bz-dC---succinyl linker---Beads

Ac-dC---Ac-dC---Ibu-dG---succinyl linker---Beads

dmf-G---dmf-G--- Bz-dC---succinyl linker---Beads

dmf-G---dmf-G--- Ibu-dG---succinyl linker---Beads

The foregoing compounds were administered directly to animals as an immunogen, without separating the oligonucleotide from the solid support, for the production of antibodies, as further described in Example 2 below.

EXAMPLE 2

Inoculation of Animals

Female BALB/c mice of eight to twelve weeks old were purchased from Charles River, Raleigh, North Carolina, USA. The mice were housed in cases with filter caps.

After oligonucleotide chain synthesis was completed as described in Example 1, the beads with nucleotides were gently crushed by hand-pressuring the glass plates, between which beads were positioned.

5 μM of each eight oligonucleotides mentioned above were mixed in 4 ml PBS (150 mM sodium chlotide in 100 mM phosphate buffer, pH 7.2).

The mixture was thoroughly vortexed suspending the crushed beads. 150 μL of the vortexed mixture was taken and added into 300 μL of PBS in a syringe. Just before injection, the solution containing beads was mixed again by shaking the syringe to suspend the broken beads. Then 150 μL or 300 μL of well-mixed solution was injected into mouse peritoneal cavity. This procedure was used for the first injection and the following boosts.

Injection time schedule:

Injection	Date (day)
first	0
second	14th
third	28th
4th	42nd
5th	56th
6th	70th
7th	84th
8th	98th
9th	112th
10th	138th
11th (final, 4 day before fusion)	142nd

Four days after the final injection, spleen cells are harvested from the animals and fused with myeloma cells (P3x.63.Ag8.653) in accordance with known techniques to produce hybridoma cell lines, which are then screened to determine the

binding characteristics as described below to isolate particular cell lines that produce the desired antibody of the invention.

EXAMPLE 3

Immunodot-Blot Assay for Antibody Characterization

The Immunodot-blot assay involves UV cross linking of oligonucleotides onto membrane paper, and is directly applicable to a test kit for detection, identification and quantifying the protecting groups on product oligomers. This procedure may be carried out as follows: *(a)* wet membrane paper with TBS (10 mM Tris, pH 7.2; 150 mM NaCl); *(b)* blot oligonucleotides to be tested onto membrane paper under vacuum; *(c)* UV cross link nucleotide onto membrane paper; *(d)* block membrane paper with 1% casein-TBST (TBS plus Tween 20, 0.1% by volume) at room temperature for 2 hr or 4° C overnight; *(e)* wash membrane with TBST 3 times, each for 15 min; *(f)* form antigen-antibody complex by incubation of plate with sample be tested (diluted in 1% casein-TBST) at room temperature for 1 hr; *(g)* wash as above; *(h)* react with second antibody conjugate (diluted in 1% casein-TBST) at room temperature for 1 hr; *(i)* wash as above; *(j)* develop color reaction by incubation of membrane with substrate solution.

EXAMPLE 4

Dot-Blot Assay of Monoclonal Antibody 1 H11

Monoclonal antibody 1 H11, produced as described in Example 2 above, was characterized by a dot-blot assay as described in Example 3 above. Results are shown as a bar graph in **Figure 1**. In Figure 1, lanes (or columns) 1 and 2 represent oligoPac-dA20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes at 4° C, respectively. Columns 3 and 4 represent oligoBz-dC20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 5 and 6 represent oligoAc-dC20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 7 and 8 represent oligoIpr-Pac-dG20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 9 and 10 represent oligoIbu-dG20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 11, 12 and 13 represent oligodT20mers, completely deprotected, with DMT group only,

and with cyanoethyl group only, respectively. Antibody activity is given as optical density (479 nm) from ELISA (Example 7 below), and the positive or negative result of the dot blot assay is given in the open or filled circle appearing over each column in the bar graph. Note the activity of monoclonal antibody 1 H11 in selectively binding to the oligoIbu-dG20mer in column 10.

EXAMPLE 5

Dot-Blot Assay of Monoclonal Antibody 7 H3

Monoclonal antibody 7 H3, produced as described in Example 2 above, was characterized by a dot-blot assay as described in Example 3 above. Results are shown as a bar graph in **Figure 1**. In Figure 1, lanes (or columns) 1 and 2 represent oligoPac-dA20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes at 4° C, respectively. Columns 3 and 4 represent oligoBz-dC20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 5 and 6 represent oligoAc-dC20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 7 and 8 represent oligoIpr-Pac-dG20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 9 and 10 represent oligoIbu-dC20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 11, 12 and 13 represent oligodT20mers, completely deprotected, with DMT group only, and with cyanoethyl group only, respectively. Antibody activity is given as optical density as described above, and the positive or negative result of the dot blot assay is given in the open or filled circle appearing over each column in the bar graph. Note the activity of monoclonal antibody 1 H11 in selectively binding to the oligoBz-dC20mer in column 4.

EXAMPLE 6

Western Blot Assay for Antibody Characterization

The Western blot assay involves low voltage transfer of oligonucleotides from gel to membrane paper and UV cross linking of oligonucleotides onto the membrane. This assay may be carried out as follows: *(a)* cast 15% non-denaturing gel containing 10 mM MgCl; *(b)* load oligonucleotides (oligomers) into the wells of the gel; *(c)* run gel at 200 voltage in ice bath; *(d)* transfer oligonucleotides from gel to membrane

paper at 25 voltage for 25 min in ice bath; *(e)* UV cross link polynucleotides on membrane; *(f)* block membrane paper with 1% casein-TBST at room temperature for 2 hr or 4° C overnight; *(g)* wash membrane with TBST 3 times, each for 15 min; *(h)* incubate samples be tested (diluted in 1% casein-TBST) at room temperature for 1 hr; *(i)* wash as above; *(j)* incubate membrane with second antibody conjugate (diluted in 1% casein-TBST) at room temperature for 1 hr; *(k)* wash as above; and *(l)* color-develop by incubation of membrane with substrate solution.

EXAMPLE 7

Detection of antibody using Biotinylated Polynucleotides as Antigen and an ELISA involving Streptavidin-Biotin System

An enzyme-linked immunosorbent assay (ELISA) for the detection of the antibody is carried out as follows: *(a)* pre-screen microtiter plate that is pre-coated with streptavidin; *(b)* coat the plate with a preparation of biotinylated oligonucleotide or other materials to be tested (at 5 μg/ml in PBS)(PBS: 150 mM NaCl, 10 mM Phosphate buffer, pH 7.4), then incubate at room temperature for 2 hrs; *(c)* wash 3 times with 0.1% Tween in PBS (PBST), each for 15 min; *(d)* block with 1% casein in PBST at room temperature for hrs or 4° C overnight; *(e)* wash as above; *(f)* form antigen-antibody complex by incubation of plate with antibody (or antibodies) at room temperature for 1 hr; *(g)* wash as above; *(h)* react with second antibody-peroxidase conjugate (in 1% casein-PBST) at room temperature for 1 hr; *(i)* wash as above; *(j)* develop color reaction by adding tetramethylbenzidine (TMB) solution (TMB solution: 42 mM TMB, 0.004% H_2O_2, 0.1 M acetate buffer, pH 5.6) and incubating at room temperature for 15 min, then stop the reaction with 2 M H_2SO_4; and *(k)* read absorption value at 469 nm.

EXAMPLE 8

ELISA and Dot-Blot Assay of Monoclonal Antibody Against Benzoyl, Isobutryl, and Isopropylphenoxyacetiyl

Monoclonal antibodies (mAb) against protecting groups benzoyl (Bz), isobutryl (ibu), and isopropylphenoxyacetiyl (ipr-Pac), produced as described in Example 2 above, were characterized by a standard ELISA assay and a dot-blot assay

as described in Example 3 above. An ELISA assay developed with biotinylated nucleic acids of 20 residues each attached to a 96-well microtiter plate demonstrated the specificity of the antibodies for their respective antigens. **Figure 3A, Figure 4A,** and **Figure 5A** show results for monoclonal antibodies against Bz, ibu, and ipr-Pac, respectively. The figures show completely deprotected (<1% Bz remaining) homopolymers of dC residues, designated oligo dC(Bz), ie. originally protected with Bz (lane 1, open bar), protected (>97% Bz remaining) oligo Bz-dC (lane 2, shaded bar), completely (< 1% ipr-Pac remaining) deprotected oligo dG(ipr-Pac) (lane 3), protected (>76% ipr-Pac) oligo ipr-PacdG (lane 4), completely (< 1% ibu remaining) deprotected oligo dG(ibu) (lane 5), protected (>91% ibu remaining) oligo ibu-dG (lane 6), and completely deprotected oligo dT (lane 7). The dT polymer had but one protecting group, dimethyltrityl (DMT) that was removed from the 5'OH of the 5'-terminal residue with mild acid. Finally, lane 8 of shows oligo dT with DMT remaining.

Dot-Blot assays of anti-Bz mAb, anti-ibu mAb, and anti-ipr-Pac mAb activities were performed in which the 20mer DNAs were linked to nitrocellulose membrane by UV. The amounts of 20mer DNA applied to the membrane are shown to the right of **Figure 3B**, **Figure 4B**, and **Figure 5B** and demonstrate the level of sensitivity of the assay. The DNAs used to test anti-Bz mAb were those described for the ELISA plus deprotected oligo dA(Bz), protected oligo Bz-dA, oligo dC(ibu), oligo ibu-dC, oligo dA(ibu) and oligo ibu-dA. **Figure 3B** shows that the anti-Bz mAb recognized the protecting group on dA and dC. The DNAs used to test anti-ibu mAB were those described for the ELISA plus protected oligo ibu-dA, deprotected oligo dA(ibu), oligo ibu-dC, oligo dC(ibu) and all are noted at the top of the dot-blot. **Figure 4B** shows that the anti-ibu mAb recognized ibu on dG, the most common use of the protecting group, but also on dA. The DNAs used to test anti-ipr-Pac mAb were those described for the ELISA plus protected oligo ibu-dA, deprotected oligo dA(ibu), oligo ibu-dC, oligo dC(ibu), oligo Bz-dA, oligo dA(Bz) and all are noted at the top of the dot-blot. **Figure 5B** shows that the anti-ipr-Pac mAb recognized ipr-Pac on dG, the most common use of the protecting group, but also on dA and dC. The mAb also recognized the ibu protecting group (ibu-dG, ibu-dA and ibu-dC). This cross-reactivity indicates that the antibody was highly selective in its identification of

a chemistry common to both ipr-Pac and ibu, possibly $CH(CH_3)_2$. Thus the anti-ibu and anti-iprPac mAbs could be used in combination to identify the protecting group remaining on an oligo.

Greater amounts of DNA were tested in a dot blot assay of anti-ibu mAb (**Figure 4C**). The results of this experiment demonstrated that the ibu protecting group was recognized by the mAb no matter which nucleobase was protected.

Figure 3C, **Figure 4D**, and **Figure 5C** demonstrate that partially deprotected oligomers can be re-treated to remove the remaining protecting groups, and re-tested with mAb. **Figure 3C** shows that anti-Bz mAb recognized re-deprotected oligomer oligo Bz-dC (center column). Likewise, **Figure 4D** shows that anti-ibu mAb recognized re-deprotected oligomer oligo ibu-dG (center column) and **Figure 5C** shows that anti-ipr-Pac mAb recognized re-deprotected oligomers oligo ipr-Pac-dG and oligo ibu-dG (columns second from left and forth from left, respectively). Thus, this approach is applicable to quality control without having to discard expensive nucleic acid samples.

An RNA standard with protecting groups Bz, ibu and ipr-Pac was synthesized and assayed for identification of the protecting groups with the mAb against Bz (**Figure 3D**), ibu (**Figure 4E**), and ipr-Pac (**Figure 5D**). Dot-blot assays clearly show that the monoclonal antibodies do not differentiate RNA from DNA. Although there was a higher background signal with RNA than with DNA, there was a significant distinction between RNA with and without protecting groups, especially at the lower amounts of RNA. The amount of RNA on the membrane was estimated from the optical absorbance of the sample.

EXAMPLE 9

mAb Dot-Blot Assay of Protecting Groups vs HPLC

Dot-blot detection of Bz groups remaining on a standardized 20mer oligo dC molecule were performed as described in Example 3. Completely deprotected and the untreated oligo dC 20mers were analyzed for the Bz protecting group using a totally independent and different quantification method. The two oligomers were hydrolyzed to the constituent nucleosides and then their nucleoside composition identified and quantified using a recognized high performance liquid chromatography (HPLC)

method with concentrated samples. Because of the lack of sensitivity, HPLC detection required 50-100 fold the amounts of Bz-dC used in the mAb assays (see **Figure 7**). **Figure 6A** shows the result of anti-Bz mAb tested against nmole amounts of Bz groups on protected oligo Bz-dC (right column) and the same nmole amounts of Bz- on Bz-dC (left column). Each amount of Bz-dC oligo was diluted with completely deprotected dC oligo of the same length (20mer) to demonstrate the sensitivity of the mAb detection even in the presence of 2500-fold dC (ie. 0.04%). The mAb assay demonstrated that the mAb could detect the Bz group on DNA even in the presence of a 2500-fold excess of dC in DNA.

The dot-blot shown in **Figure 6A** was subjected to densitometry to quantitate the mAb response. After background subtraction, the remaining density was plotted as a function of Bz groups in oligo Bz-dC determined by HPLC (**Figure 6B**). The data indicated that the high sensitivity of the anti Bz mAb detection was linear in 0.1-1.0 nmol range.

Next, it was determined whether the mAb response could be enhanced with an increase in the amount of DNA on the dot-blot membrane. The amount of Bz was determined by standard HPLC methods. This experiment showed that detection of the Bz protecting group in a mixture of the protected sample with the deprotected sample at a ratio of 1/2500 could be enhanced by increasing the amount of DNA on the membrane, though the ratio was maintained (**Figure 6C**).

Finally, experiments were conducted to show a direct comparison of the mAb and HPLC detection of Bz. Anti-Bz mAb was utilized in a dot-blot assay to detect Bz on dC in the oligo Bz-dC (20mer). The density response of the Bz group detected Bz by the mAb assay and quantified by densitometry was plotted against the amount of Bz in the DNA on each dot (**Figure 7A**). The amount of Bz in the DNA was calibrated by digestion of a large amount of DNA and analysis by HPLC identification and quantification of the Bz-dC mononucleoside. For HPLC experiments, three samples of Bz-dC oligo were hydrolyzed and analyzed for composition by HPLC. The response of the UV-diode array detector was plotted against the amount of Bz in the samples (**Figure 7B**). The sample amounts were determined by comparison to samples "spiked" with known amounts of Bz-dC. The amounts of Bz-dC added to samples as spikes were from a weighed stock of Bz-dC.

Thus, the HPLC response was calibrated with known amounts of Bz-dC. The results of these experiments show that the detection of Bz by anti-Bz mAb was within the pmole range whereas HPLC detection of Bz was limited to the nmole range.

EXAMPLE 10

Detection of Remaining Protecting Groups in Commercial Samples

A blind study was conducted to demonstrate the detection of remaining protecting groups in commercial samples by mAb. The purpose of the this experiment was to determine if protecting groups could be detected and identified with mAb technology in presumably completely deprotected samples that had been treated as commonly accomplished in the oligo synthesis industry. The nature of the protecting groups used by eight selected companies was not known, thus the experiment was a blind study. Two 20mer oligos (oligo dA-dC and oligo dG-dT) from each of the eight companies were ordered to be synthesized and deprotected, and salt removed under as identical conditions as possible. The oligos were shipped by express mail, as is often the case, and then subjected to mAb analysis by dot blot. The dA-dC oligo from one company (#6), and possibly a second (#2), had remaining Bz protecting groups as determined by anti-Bz mAb testing (**Figure 8A**). The dG-dT oligos from two companies (#2 and #6) had ipr-Pac protecting groups remaining as determined by anti-ipr-Pac mAb (**Figure 8B**). The remaining protecting groups in the commercial samples were confirmed by increasing amounts of sample and further deprotection and re-analyses. The oligo dA-dC samples from companies #2 and #6 were tested in higher amounts to confirm the presence of the Bz protecting group. In addition, the samples were treated to remove the remaining protecting groups using a standard protocol. The re-analysis after further deprotection indicated that the groups were now removed (**Figure 8C**). This also demonstrates that expensive nucleic acid samples can be re-treated to remove protecting groups and that they need not be discarded. The oligo dG-dT samples were re-treated to remove remaining protecting groups and re-analyzed with anti-ipr-Pac mAb with the result that the ipr-Pac group could be removed without sacrificing the DNA (**Figure 8D**).

EXAMPLE 11

Polyclonal Antibody Against Dimethyltrityl

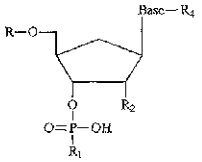
Production and analyses of polyclonal antibody against the 5' terminal protecting group, dimethyltrityl (DMT) were as described in Example 2. Four mice were inoculated with DMT and sera were drawn from the mice after some weeks of boosting with antigen. DMT [DMT—OH], three DMT at the 5'-end of the deoxynucleotide trimer $d(T)_3$ [$(DMT)_3$-$d(T)_3$], three DMT at the 5'-end of the deoxynucleotide 20mer $d(T)_3$ with 3'-biotin [$(DMT)_3$-$d(T)_{20}$-biotin], one DMT at the 5'-end of the deoxynucleotide 20mer $d(T)_{20}$ with 3'-biotin [DMT-$d(T)_{20}$-biotin], the dT 20mer with 3'-biotin [$d(T)_{20}$-biotin], one DMT with biotin [DMT-biotin] and tris-borate saline control were applied to a nitrocellulose membrane that was then assayed with mouse sera (inoculated mice #1-4 and a control serum, normal) to assess anti-DMT antibody, mild acid to reveal presence of the DMT (TBS), and avidin to reveal the presence of biotin (Figure 9). Sera from mice #2 and #4 recognized DMT [as $(DMT)_3$-$d(T)_3$], whereas mice #1, #3, and the normal mouse did not. Mild acid revealed the presence of DMT as a yellow color (not visible in figure) and avidin revealed the presence of biotin.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. An antibody that specifically binds to a synthetic oligonucleotide having a organic protecting group covalently bound thereto, which antibody does not bind to said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto.

2. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I):



(I)

wherein:

R is H or a protecting group;

subject to the proviso that R is a covalent bond to an adjacent nucleotide when said protected base is not a 5' terminal nucleotide in said oligonucleotide;

R_1 is H or a protecting group;

subject to the proviso that R_1 is a covalent bond to an adjacent nucleotide when said protected base is not a 3' terminal nucleotide in said oligonucleotide;

R_2 is H or $-OR_3$;

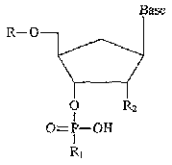
R_3 is H or a protecting group;

Base is a purine or pyrimidine base;

R_4 is a protecting group bonded to an amino group of said base;

and further subject to the proviso that when one of R, R_1, R_3 and R_4 is a protecting group, then the others of R, R_1, R_3 and R_4 are not protecting groups.

3. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides and has a 5' nucleotide, and wherein said 5' nucleotide is a protected nucleotide according to Formula (I):



(I)

wherein:

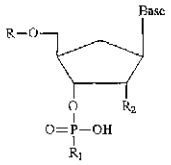
R is a protecting group;

R_1 is a covalent bond to an adjacent nucleotide;

R_2 is -H or –OH; and

Base is a purine or pyrimidine base.

4. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides and has a 3' nucleotide, and wherein said 3' nucleotide is a protected nucleotide according to Formula (I):



(I)

wherein:

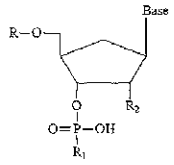
R is a covalent bond to an adjacent nucleotide;

R_1 is a protecting group;

R_2 is H or –OH; and

Base is a purine or pyrimidine base.

5. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I):



(I)

wherein:

R is a covalent bond to an adjacent nucleotide;

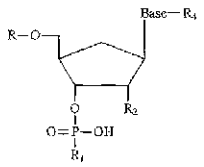
R_1 is a covalent bond to an adjacent nucleotide;

R_2 is $-OR_3$;

R_3 a protecting group; and

Base is a purine or pyrimidine base.

6. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I):



(I)

wherein:

R is a covalent bond to an adjacent nucleotide;

R_1 is a covalent bond to an adjacent nucleotide;

R_2 is H or —OH;

Base is a purine or pyrimidine base; and

R_4 is a protecting group bonded to an amino group of said base.

7. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected with a photolabile protecting group.

8. An antibody according to claim 1, which antibody is a polyclonal antibody.

9. An antibody according to claim 1, which antibody is a monoclonal antibody.

10. An antibody according to claim 1 immobilized on a solid support.

11. A cell that expresses an antibody according to claim 9.

12. A cell according to claim 11, which cell is a hybridoma.

13. A cell according to claim 11, which cell contains and expresses a heterologous nucleic acid encoding said antibody.

14. A method for detecting incomplete deprotection of a synthetic oligonucleotide by immunoassay, said immunoassay comprising the steps of:

contacting a synthetic oligonucleotide to an antibody according to claim 1; and then

detecting the presence or absence of binding of said antibody to said oligonucleotide, the presence of binding indicating incomplete deprotection of said synthetic oligonucleotide.

15. A method according to claim 14, wherein said immunoassay is a heterogeneous immunoassay.

16. A method according to claim 14, wherein said immunoassay is a homogeneous immunoassay.

17. A method according to claim 14, wherein said immunoassay is a sandwich assay.

18. A method according to claim 14, wherein said oligonucleotide is immobilized on a solid support.

19. A method for separating protected from fully deprotected synthetic oligonucleotides, comprising:

contacting a mixture of protected from fully deprotected synthetic oligonucleotides to antibodies according to claim 1, wherein said protected synthetic oligonucleotides have said organic protecting group covalently bound thereto, so that said protected synthetic oligonucleotides bind to said antibody; and then

separating said antibodies from said fully deprotected synthetic oligonucleotides.

20. A method according to claim 19, wherein said antibody is immobilized on a solid support.

21. A method according to claim 19, wherein said protected synthetic oligonucleotide is a partially protected synthetic oligonucleotide.

22. A method according to claim 19, wherein said contacting and separating steps are carried out by affinity chromatography.

23. An article useful for the determining incomplete deprotection of a synthetic oligonucleotide in an immunoassay, said article comprising:

a solid support having a surface portion, said surface portion having at least two separate discrete regions formed thereon;

a first oligonucleotide bound to one of said separate discrete regions, said first oligonucleotide having a protecting group bound thereto; and

a second oligonucleotide bound to another of said separate discrete regions, said second oligonucleotide not having said protecting group bound thereto;

wherein the nucleotide sequence of said first and second oligonucleotides are the same.

24. An article according to claim 23, further comprising:

a third oligonucleotide bound to another of said separate discrete regions; said third oligonucleotide also having said protecting group bound to said first oligonucleotide bound thereto;

wherein said third oligonucleotide is partially deprotected;

and wherein the nucleotide sequence of said first, second, and third oligonucleotides are the same.

25. An article according to claim 23, wherein said substrate comprises a nitrocellulose strip.

26. A method of making an antibody that specifically binds to a synthetic oligonucleotide having a organic protecting group covalently bound thereto, which antibody does not bind to the said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto, said method comprising the steps of:

synthesizing said synthetic oligonucleotide on a solid particulate support with said organic protecting group covalently bound to said synthetic oligonucleotide, or synthesizing a nucleotide on said solid support with said organic protecting group bound to said nucleotide; and then, without removing said oligonucleotide or nucleotide from said solid support;

immunizing an animal with said synthetic oligonucleotide or nucleotide bound to said solid support in an amount sufficient to produce said antibody.

27. A method according to claim 26, wherein said synthesizing step is followed by the step of fragmenting said beads prior to said immunizing step.

28. A method according to claim 26, further comprising the step of:

collecting said antibody from said animal.

29. A method according to claim 26, further comprising the steps of:

collecting spleen cells from said animal; then

producing a plurality of hybridoma cell lines from said spleen cells; and then

isolating a particular hybridoma cell line that produces said antibody from said plurality of hybridoma cell lines.

30. A method according to claim 26, wherein said synthetic oligonucleotide is covalently bound to said solid support.

31. A method according to claim 26, wherein said synthetic oligonucleotide is covalently bound to said solid support with a succinyl linker.

32. A method according to claim 26, wherein said solid support comprises a controlled pore glass bead.

33. A method of screening an oligonucleotide array for insufficient deprotection or insufficient elongation of oligonucleotides therein, said method comprising the steps of:

(a) providing an oligonucleotide array comprising a substrate having a plurality of different oligonucleotides immobilized thereon, with said different oligonucleotides immobilized in different separate and discrete locations on said substrate;

(b) providing an antibody that specifically binds to a synthetic oligonucleotide having an organic protecting group covalently bound thereto, which antibody does not bind to said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto; and then

(c) contacting said antibody to said oligonucleotide array to thereby detect the presence or absence of binding of said antibody selected and discrete locations on

said array, the presence of binding to separate and discrete locations in said array indicating insufficient deprotection or insufficient elongation of oligonucleotides therein.

34. A method according to claim 33, wherein said substrate comprises silicon.

35. A method according to claim 33, wherein said step of providing an array is carried out by synthesizing said oligonucleotides *in situ* on said substrate.

36. A method according to claim 33, further comprising repeating steps *(b)* to *(c)* at least once with a different antibody on each repetition so that a plurality of different protecting groups on oligonucleotides in the array may be detected.

37. A method according to claim 33, further comprising the step of:

generating an indicia recording the presence of insufficient deprotection or insufficient elongation of oligonucleotides in at least one separate and discrete location on said array.

38. A method according to claim 37, wherein said indicia is a qualitative indicia.

39. A method according to claim 37, wherein said indicia is a quantitative indicia.

40. A correctable oligonucleotide array, comprising, in combination:

(a) a substrate having a plurality of different oligonucleotides immobilized thereon, with said different oligonucleotides immobilized in different separate and discrete locations on said substrate; and

(b) a plurality of indicia associated with said array, said indicia recording the presence of insufficient deprotection or insufficient elongation of at least one oligonucleotide, each of said at least one oligonucleotide located in a different separate and discrete location on said array.

41. An array according to claim 40, wherein said substrate has at least 1000 different oligonucleotides immobilized in different separate and discrete locations on said substrate.

42. An array according to claim 40, wherein said indicia are stored in or printed on said array.

43. An array according to claim 40, wherein said indicia are contained in a computer file, said array further comprising an identifier associating said substrate and said indicia.

44. An array according to claim 40, wherein said indicia are contained on a web site, said array further comprising an identifier associating said substrate and said indicia.

45. A method of using an oligonucleotide array and compensating for insufficient deprotection or insufficient elongation of oligonucleotides on said array, comprising the steps of:

(a) providing a substrate having a plurality of different oligonucleotides immobilized thereon, with said different oligonucleotides immobilized in different separate and discrete locations on said substrate;

(b) providing indicia associated with said array, said indicia recording the presence of insufficient deprotection or insufficient elongation of at least one oligonucleotide, said at least one oligonucleotide located in a separate and discrete locations on said array;

(c) providing a test compound;

(d) detecting the binding of said test compound to at least one of said plurality of different oligonucleotides; and then

(d) determining the degree of binding of said test compound to said oligonucleotide from *(i)* said detected binding and *(ii)* said indicia recording the presence of insufficient deprotection or insufficient elongation, so that said

insufficient deprotection or insufficient elongation is compensated for during said determining step.

46. A method according to claim 45, wherein said test compound is a protein, peptide, or oligonucleotide.

47. A method according to claim 45, wherein said test compound is mRNA.

48. A method according to claim 45, wherein said determining step is carried out by generating a color indication of degree of binding.

49. A method according to claim 45, wherein said determining step is carried out by generating a numeric indication of degree of binding.

50. A method according to claim 45, wherein said degree of binding is binding affinity, binding amount, or both binding affinity and binding amount.

51. A method of using an oligonucleotide array while compensating for insufficient deprotection or insufficient elongation of oligonucleotides on said array, said method comprising the steps of:

(a) providing a substrate having a plurality of different oligonucleotides immobilized thereon, with said different oligonucleotides immobilized in different separate and discrete locations on said substrate;

(b) providing indicia associated with said array, said indicia recording the presence of insufficient deprotection or insufficient elongation of at least one oligonucleotide, said at least one oligonucleotide located in a separate and discrete locations on said array;

(c) providing a test compound;

(d) contacting said test compound to said array;

(d) deleting from analysis said at least one oligonucleotide in a separate and discrete location having insufficient deprotection, with binding of said test compound

to said array being detected with the remaining oligonucleotides in separate and discrete locations that have not been deleted from analysis; and then

(d) detecting the binding of said test compound to said remaining oligonucleotides in separate and discrete locations in said array.

52. A method according to claim 51, wherein said test compound is a protein, peptide, or oligonucleotide.

53. A method according to claim 51, wherein said test compound is mRNA.

54. A method according to claim 51, wherein said detecting step is carried out by generating a color indication of binding.

55. A method according to claim 51, wherein said detecting step is carried out by generating a numeric indication of binding.

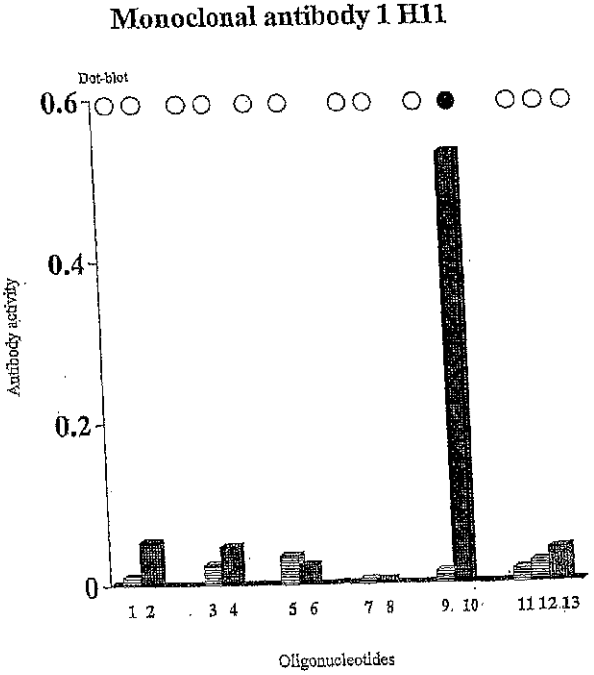

Monoclonal antibody 1 H11
Dot-blot
0.6
0.4
0.2
0
Antibody activity
1 2 3 4 5 6 7 8 9. 10 11 12 13
Oligonucleotides

FIG 1

Monoclonal antibody 7 H3

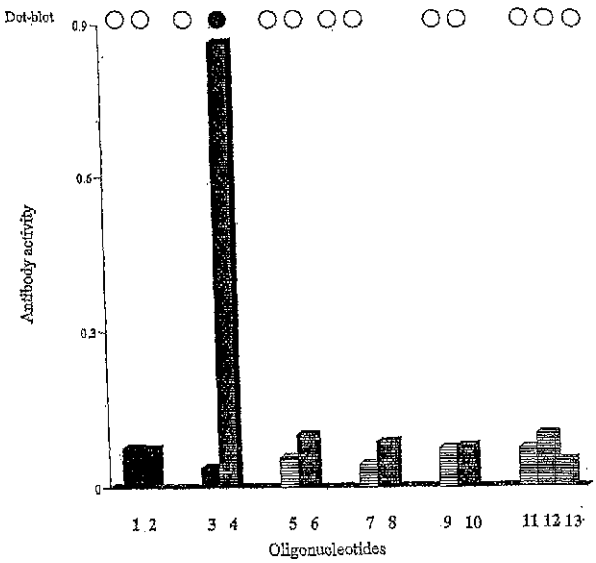


FIG 2

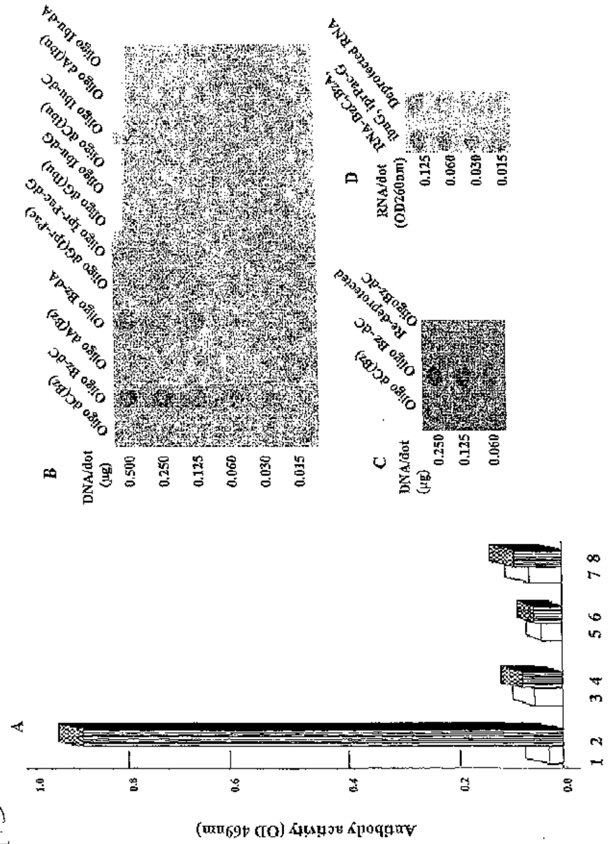

A
Antibody activity (OD 469nm)
1.0
0.8
0.6
0.4
0.2
0.0
1 2
3 4
5 6
7 8
B
DNA/dot (μg)
0.500
0.250
0.125
0.060
0.030
0.015
Oligo dC(Bz)
Oligo Bz-dC
Oligo dA(Bz)
Oligo Bz-dA
Oligo dG(ipr-Pac)
Oligo ipr-Pac-dG
Oligo dG(ibu)
Oligo ibu-dG
Oligo dC(ibu)
Oligo ibu-dC
Oligo dA(ibu)
Oligo ibu-dA
C
DNA/dot (μg)
0.250
0.125
0.060
Oligo dC(Bz)
Oligo Bz-dC
Re-deprotected Oligobz-dC
D
RNA/dot (OD260nm)
0.125
0.060
0.030
0.015
RNA-BzC, BzA, ibuG, iprPac-G
Deprotected RNA

Fig. 3

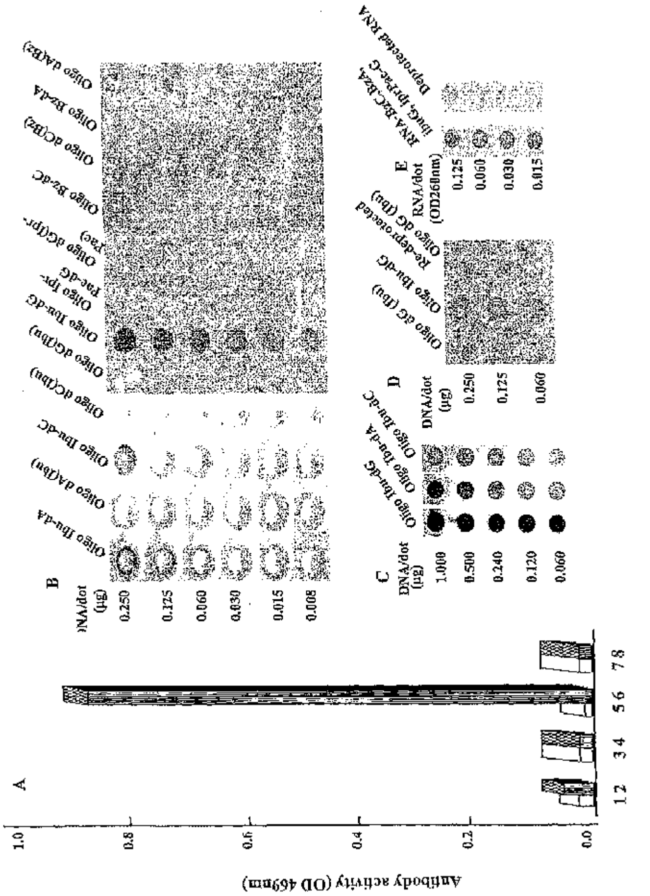


Fig.4

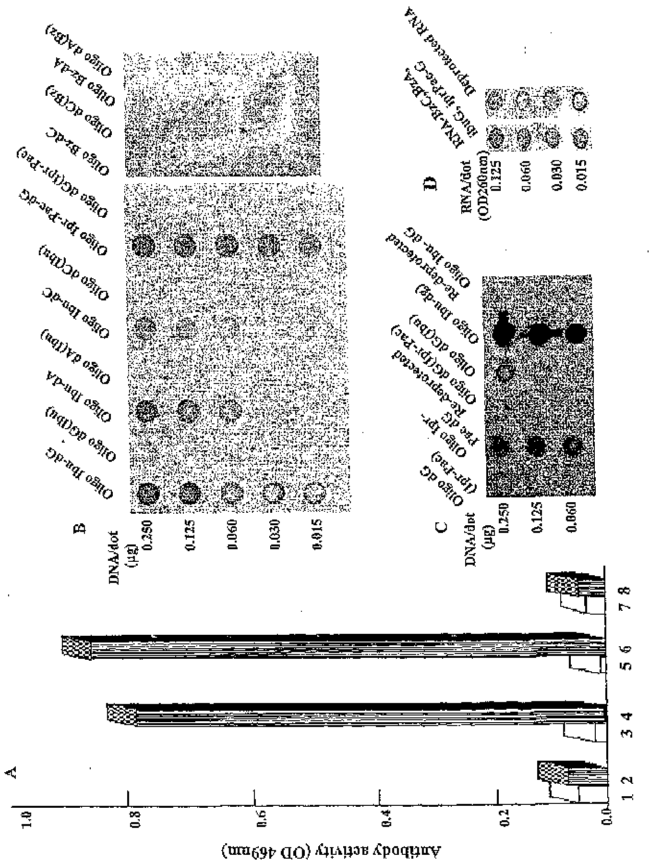

A
Antibody activity (OD 469nm)
1.0
0.8
0.6
0.4
0.2
0.0
1 2
3 4
5 6
7 8
B
DNA/dot (μg)
0.250
0.125
0.060
0.030
0.015
Oligo Ibu-dG
Oligo dG(Ibu)
Oligo Ibu-dA
Oligo dA(Ibu)
Oligo Ibu-dC
Oligo dC(Ibu)
Oligo Ipr-Pac-dG
Oligo dG(Ipr-Pac)
Oligo Bz-dC
Oligo dC(Bz)
Oligo Bz-dA
Oligo dA(Bz)
C
DNA/dot (μg)
0.250
0.125
0.060
Oligo dG (Ipr-Pac)
Oligo Ipr-Pac dG
Re-deprotected Oligo dG(Ipr-Pac)
Oligo dC(Ibu)
Oligo Ibu-dG Re-deprotected Oligo Ibu-dG
D
RNA/dot (OD260nm)
0.125
0.060
0.030
0.015
RNA-BzC,BzA, IbuG, iprPac-G
Deprotected RNA

Fig. 5

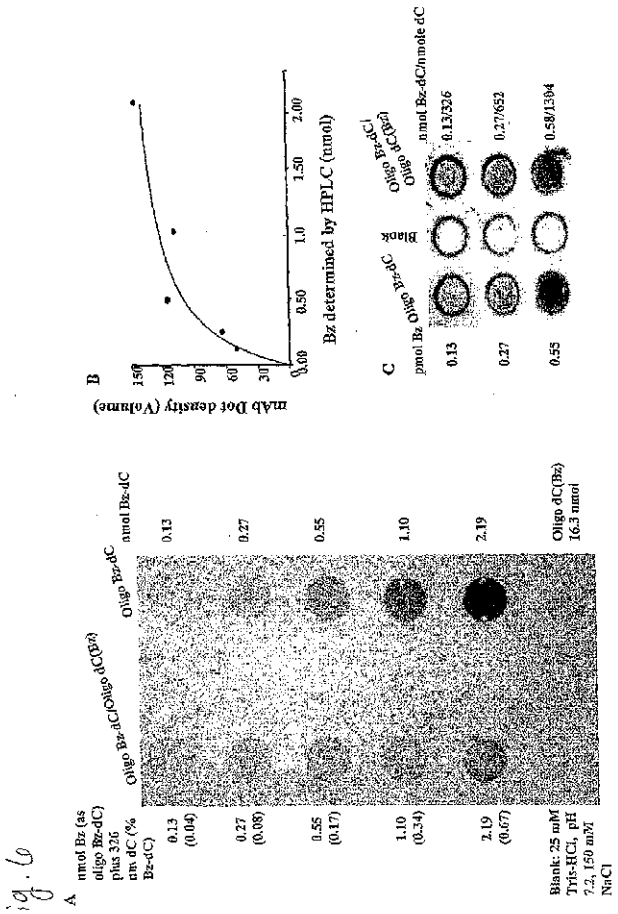

A
nmol Bz (as oligo Bz-dC) plus 326 nm dC (% Bz-dC)
0.13 (0.04)
0.27 (0.08)
0.55 (0.17)
1.10 (0.34)
2.19 (0.67)
Blank: 25 mM Tris-HCl, pH 7.2, 150 mM NaCl
Oligo Bz-dC/Oligo dC(Bz)
Oligo Bz-dC
nmol Bz-dC
0.13
0.27
0.55
1.10
2.19
Oligo dC(Bz) 16.3 nmol
B
mAb Dot density (Volume)
150
120
90
60
30
0.00
0.50
1.0
1.50
2.00
Bz determined by HPLC (nmol)
C
pmol Bz Oligo Bz-dC
Blank
Oligo Bz-dC/Oligo dC(Bz)
nmol Bz-dC/nmole dC
0.13
0.27
0.55
0.13/326
0.27/652
0.58/1304

Fig. 6

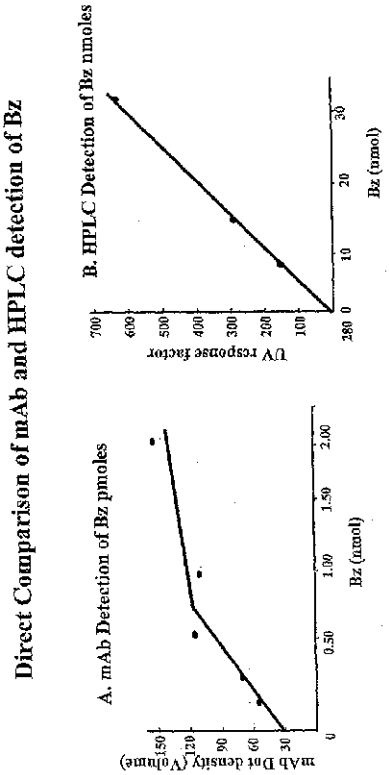
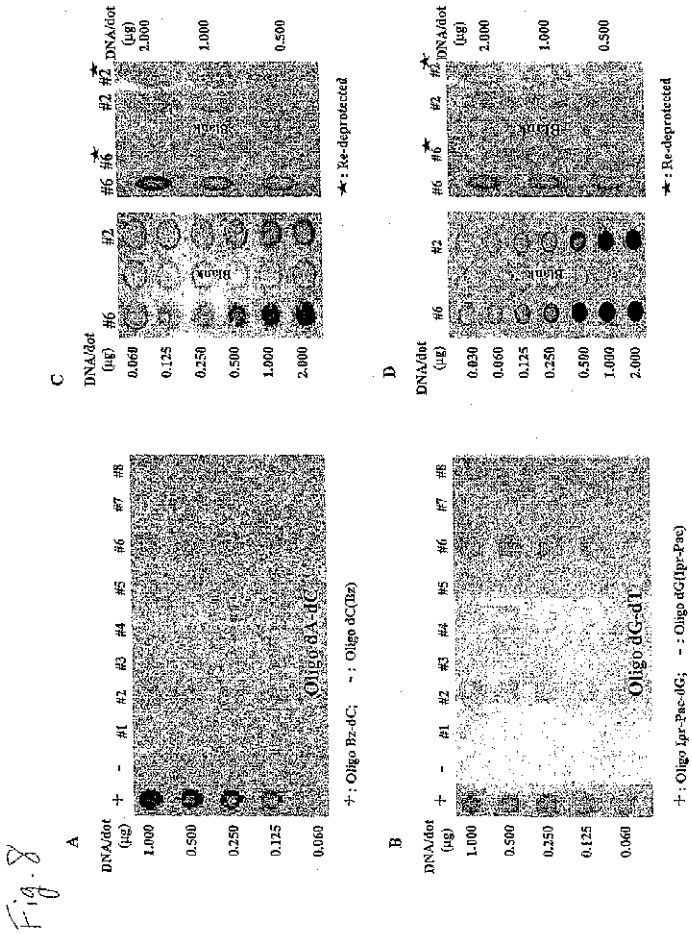

Direct Comparison of mAb and HPLC detection of Bz
A. mAb Detection of Bz pmoles
mAb Dot density (Volume)
150
120
90
60
30
0
0.50
1.00
1.50
2.00
Bz (nmol)
B. HPLC Detection of Bz nmoles
UV response factor
700
600
500
400
300
200
100
280 0
10
20
30
Bz (nmol)

Fig. 7.

Fig. 8


A
DNA/dot (μg)
1.000
0.500
0.250
0.125
0.060
+ - #1 #2 #3 #4 #5 #6 #7 #8
Oligo dA-dC
+ : Oligo Bz-dC; - : Oligo dC(Bz)
B
DNA/dot (μg)
1.000
0.500
0.250
0.125
0.060
+ - #1 #2 #3 #4 #5 #6 #7 #8
Oligo dG-dT
+ : Oligo Ipr-Pac-dG; - : Oligo dG(Ipr-Pac)
C
DNA/dot (μg)
0.060
0.125
0.250
0.500
1.000
2.000
#6 #2
Bz-dC
#6 #6★ #2 #2★ DNA/dot (μg)
2.000
1.000
0.500
★: Re-deprotected
D
DNA/dot (μg)
0.030
0.060
0.125
0.250
0.500
1.000
2.000
#6 #2
Ipr-Pac-dG
#6 #6★ #2 #2★ DNA/dot (μg)
2.000
1.000
0.500
★: Re-deprotected

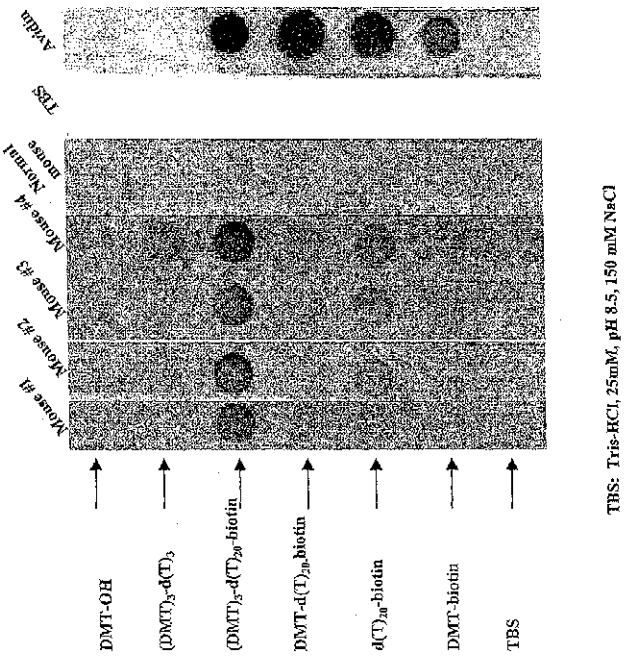

Avidin
TBS
Mouse
Normal
Mouse #4
Mouse #3
Mouse #2
Mouse #1
DMT-OH
(DMT)5-d(T)5
(DMT)5-d(T)20-biotin
DMT-d(T)20-biotin
d(T)20-biotin
DMT-biotin
TBS
TBS: Tris-HCl, 25mM, pH 8.5, 150 mM NaCl

Fig. 9

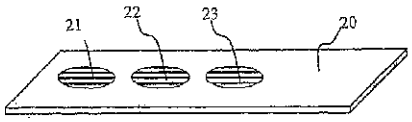


Figure 10

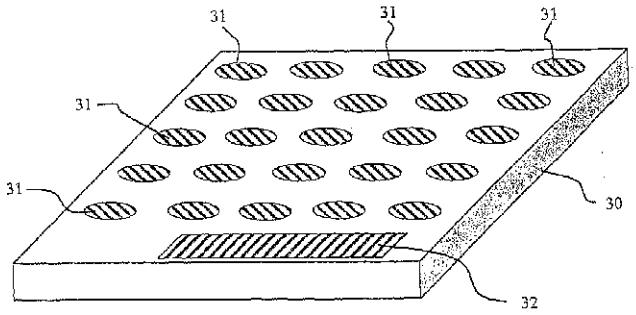


Figure 11

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(54) Title: METHODS AND COMPOSITIONS FOR DETERMINING THE PURITY OF CHEMICALLY SYNTHESIZED NUCLEIC ACIDS

(57) Abstract: This application describes an antibody that specifically binds to a synthetic oligomer (e.g., an oligonucleotide or oligopeptide) having a organic protecting group covalently bound thereto, which antibody does not bind to that synthetic oligomer when the organic protecting group is not covalently bound thereto. Methods of making and using such antibodies are also disclosed, along with cells for making such antibodies and articles carrying immobilized oligomers that can be used in assay procedures with such antibodies.

METHODS AND COMPOSITIONS FOR DETERMINING THE PURITY OF CHEMICALLY SYNTHESIZED NUCLEIC ACIDS

Paul F. Agris, Christopher D. J. Pearce, and Lloyd G. Mitchell

Related Applications

This application is a continuation-in-part of commonly owned, copending application Serial No. 09/476,975, filed December 31, 1999, the disclosure of which is incorporated by reference herein in its entirety.

Field of the Invention

The present invention concerns the detection, identification and quantification of protecting groups remaining after chemical synthesis of oligomers, particularly oligonucleotides.

Background of the Invention

Over the past decade automated chemical synthesis of nucleic acids such as DNA and RNA on solid supports has been developed. These chemical processes include the use of agents to protect the exocyclic amines of the nucleotide bases adenine, thymine, cytosine and guanine and to direct the synthesis by blocking the 2'OH of RNA's ribose. The bases within the nucleic acid product of the synthesis are deprotected upon cleavage of the nucleic acid from the solid support. However, the extent of base deprotection is not easily determined.

For example, after base deprotection of synthetic RNA, products still contain the 2'-dimethylsilyl *tert*-butyl group as a protection of the 2'OH of the ribose moiety. This protecting group is removed carefully by chemical means so as not to effect the chemistry and structure of the RNA. However, the extent of deprotection of the 2'OH is not readily determined. The nucleic acid is purified by high pressure liquid chromatography or by gel electrophoresis. However, some of the unwanted products

of the synthesis are complete nucleic acid sequences that still contain one or more protecting groups, and shorter than full length (aborted) sequences difficult to separate from full length sequences, especially for oligomers of longer than 50 nucleosides. At present, there is no easy method to determine how much of each protecting group, if any, still remains on the product, and what proportion of the product is full-length. *See generally* Davis, G.E., Gehrke, C.W., Kuo, K.C., and Agris, P.F. (1979) Major and Modified Nucleosides in tRNA Hydrolysates by High Performance Liquid Chromatography. *J. Chromatogr.* 173:281-298; Agris, P.F., Tompson, J.G., Gehrke, C.W., Kuo, K.C., and Rice, R.H. (1980) High-Performance Liquid Chromatography and Mass Spectrometry of Transfer RNA Bases for Isotopic Abundance. *J. Chromatogr.* **194**:205-212; Gehrke, C.W., Kuo, K.C., McCune, R.A., Gerhardt, K.O., and Agris, P.F. (1981) Quantitative Enzymatic Hydrolysis of tRNAs: RP-HPLC of tRNA Nucleosides. *J. Chromatogr.* **230**:297-308; Chromatography and Modification of Nucleosides Volumes A, B and C (Gehrke, C.W. and Kuo, K.C.T., eds.), Elsevier Publishing Co. 1990; Agris, P.F. and Sierzputowska-Gracz, H. (1990) Three Dimensional Dynamic Structure of tRNA's by Nuclear Magnetic Resonance. In Chromatography and Modification of Nucleosides (Gehrke, C.W. and Kuo, K.C.T., eds.), Elsevier Publishing Co., pp. 225-253; Agris, P.F., Hayden, J., Sierzputowska-Gracz, H., Ditson, S., Degres, J.A., Tempesta, M., Kuo, K.C. and Gehrke, C.W. (1990) Compendium on Biological, Biochemical, Chemical, Physical and Spectroscopic Properties of RNA and DNA Nucleosides. In Chromatography and Modification of Nucleosides, Elsevier Publishing Co.

The incomplete removal of the protecting group and lack of a simple assay is a problem for two industries and for numerous researchers world wide: *(i)* the multitude of companies now providing nucleic acid sequence synthesis products by overnight delivery have difficulty telling their customers the extent to which the product is deprotected; *(ii)* pharmaceutical companies cannot easily verify for regulatory agencies the purity and/or length of the therapeutic or diagnostic oligonucleotide products they seek to introduce or market. Accordingly, there is a need for simple and reliable techniques for determining the purity and proportion of full length of oligonucleotide products.

Summary of the Invention

A first aspect of the present invention is an antibody (*e.g.*, a monoclonal or polyclonal antibody) that specifically binds to a synthetic oligomer (*i.e.*, an

oligonucleotide or oligopeptide) having a organic protecting group covalently bound thereto, which antibody does not bind to that synthetic oligomer when the organic protecting group is not covalently bound thereto.

A second aspect of the present invention comprises a cell or cells, including cell cultures and isolated cells, that express an antibody as described above. Such cells include hybridoma cells, as well as recombinant cells that contain and express a heterologous nucleic acid encoding the antibody.

A third aspect of the present invention is a method for detecting incomplete deprotection of a synthetic oligomer by immunoassay, said immunoassay comprising the steps of: *(a)* contacting a synthetic oligomer to an antibody as described above, and then *(b)* detecting the presence or absence of binding of said antibody to said oligomer, the presence of binding indicating incomplete deprotection of said synthetic oligomer. Any suitable assay format can be employed, including heterogeneous and homogeneous immunoassays. For example, the immunoassay may be an immunoblot-dot assay, or may be a sandwich assay.

A fourth aspect of the present invention is a method for separating protected (including partially and completely protected) synthetic oligomers from fully deprotected synthetic oligomers. The method comprises *(a)* contacting a mixture of protected from fully deprotected synthetic oligomers to antibodies as described above, wherein the protected synthetic oligomers have the organic protecting group covalently bound thereto, so that the protected synthetic oligomers bind to the antibody; and then separating the antibodies from the fully deprotected oligomers. The antibody may be immobilized on a solid support to facilitate separation. The protected synthetic oligomer may be a partially protected synthetic oligomer (for which one application is the identification and/or purification of full-length versus aborted sequence oligomers) or a fully protected synthetic oligomer that has not undergone deprotection. Any separation format may be used, including but not limited to affinity chromatography.

A fifth aspect of the invention is an article useful for the determining incomplete deprotection of a synthetic oligomer in an immunoassay, said article comprising: *(a)* a solid support (*e.g.*, a nitrocellulose strip) having a surface portion, said surface portion having at least two separate discrete regions formed thereon; *(b)* a

first oligomer bound to one of said separate discrete regions, said first oligomer having a protecting group bound thereto; and *(c)* a second oligomer bound to another of said separate discrete regions, said second oligomer not having said protecting group bound thereto; wherein the nucleotide sequence of said first and second oligomers are the same. In a preferred embodiment, the article further comprises *(d)*a third oligomer bound to another of said separate discrete regions; said third oligomer also having said protecting group bound to said first oligomer bound thereto; wherein said third oligomer is partially deprotected; and wherein the nucleotide sequence of said first, second, and third oligomers are the same.

A sixth aspect of the present invention is a method of making an antibody that specifically binds to a synthetic oligomer having a organic protecting group covalently bound thereto, which antibody does not bind to the said synthetic oligomer when said organic protecting group is not covalently bound thereto, said method comprising the steps of: *(a)* synthesizing said synthetic oligomer on a solid particulate support (and preferably covalently bound thereto, *e.g.*, with a succinyl linker) with said organic protecting group covalently bound to said synthetic oligomer (or synthesizing a monomer of a single nucleotide on the solid support, with the single nucleotide having said protecting group covalently bound thereto); and then, without removing said oligomer from said solid support; *(b)* immunizing an animal with said synthetic oligomer bound to said solid support (or monomer bound to said solid support) in an amount sufficient to produce said antibody. Optionally, the solid support can be replaced with a carrier group such as a protein (e.g., bovine serum albumin).

In summary, the antibodies and methods of the present invention are useful in immunoassays, such as for the qualitative and quantitative detection of protecting groups used in organic synthetic processes, with particular application to oligonucleotides or peptides in research, therapeutics, diagnostics and biomedical science. The antibodies of the invention can be used in purification techniques, such as for the separation of final products from by-product contaminants. The instant invention can be used in the course of quality control of oligonucleotide and peptide synthesis, such as in the quality control of drugs for gene therapy, antisense, antigene and control of gene expression, in the quality control of biomedical polymers that may

contain protecting groups, and as probes for purification and characterization of synthetic oligomers, particularly oligonucleotides or peptides.

The present invention is explained in greater detail in the drawings herein and the specification set forth below.

Brief Description of the Drawings

Figure 1 is a dot-blot immunoassay of monoclonal antibody 1 H11, which selectively binds to oligoIbu-dG20mers.

Figure 2 is a dot-blot immunoassay of monoclonal antibody 7H3, which selectively binds to oligoBz-dC20mers.

Figure 3 shows ELISA (**A**) and dot-blot (**B**) results demonstrating specificity and detection sensitivity of a monoclonal antibody (mAb) of the commonly used protecting group, benzoyl (Bz), for the chemical synthesis of nucleic acids. Partially deprotected oligomer oligo Bz-dC (center column) can be re-treated to remove the remaining protecting groups, and re-tested with mAb (**C**). An RNA standard with protecting groups Bz, ibu and ipr-Pac was synthesized and assayed for identification of the protecting groups with the mAb against Bz (**D**).

Figure 4 shows ELISA (**A**) and dot-blot (**B**) results demonstrating specificity and sensitivity of a monoclonal antibody (mAb) and its detection of the commonly used protecting group, isobutryl (ibu), for the chemical synthesis of nucleic acids. Dot-blot assay with high amounts of DNA demonstrates that the ibu protecting group was recognized by the mAb no matter which nucleobase was protected (**C**). Partially deprotected oligomer oligo Bz-dC (center column) can be re-treated to remove the remaining protecting groups, and re-tested with mAb (**D**). An RNA standard with protecting groups Bz, ibu and ipr-Pac was synthesized and assayed for identification of the protecting groups with the mAb against ibu (**E**).

Figure 5 shows ELISA (**A**) and dot-blot (**B**) results demonstrating specificity and sensitivity of a monoclonal antibody (mAb) and its detection of the commonly used protecting group, isopropylphenoxyacetyl (ipr-Pac), for the chemical synthesis of nucleic acids. Partially deprotected oligomers oligo ibr-Pac-dG and oligo ibu-dG (columns second from left and forth from left, respectively) can be re-treated to remove the remaining protecting groups, and re-tested with mAb (**C**). An RNA

standard with protecting groups Bz, ibu and ipr-Pac was synthesized and assayed for identification of the protecting groups with the mAb against ipr-Pac (**D**).

Figure 6 shows a mAb dot-blot assay of protecting groups demonstrating the sensitivity and quantifiable response of the technology as related to HPLC. Dot-blot detection of Bz groups remaining on a standardized 20mer oligo dC molecule was analzyed (**A**) and a quantitation of the mAb response (**B**) was determined. The mAb response was analyzed with an increase in the amount of DNA on the dot-blot membrane (**C**). The column on the left is just the protected Bz-dC 20mer. The column on the right is the protected Bz-dC together with a 2500-fold excess of the completely deprotected oligo dC(Bz).

Figure 7 shows a direct comparison of the mAb and HPLC detection of Bz in the pmole (**A**) and nmol range (**B**), respectively.

Figure 8 shows a blind study demonstrating the detection of remaining protecting groups in commercial samples. dA-dC oligos were analyzed with anti-Bz mAb (**A**) and dG-dT oligos were analyzed with anti-ipr-Pac mAb (**B**). The oligo dA-dC samples from companies #2 and #6 were tested in higher amounts to confirm the presence of the Bz protecting group (**C**). In addition, the samples were treated to remove the remaining protecting groups using a standard protocol. The oligo dG-dT samples were assayed for the ipr-Pac protecting groups (**D**). The samples were re-treated to remove remaining protecting groups and re-analyzed as in (C).

Figure 9 shows the production and analyses of polycolonal antibody against the 5'terminal protecting group, dimethyltrityl (DMT).

Figure 10 shows a substrate carrying different oligonucleotides of the same sequence, but with varying degrees of deprotection, that may be used as a testing standard to screen similar oligonucleotides of the same sequence for varying degrees of protection or deprotection.

Figure 11 illustrates an oligonucleotide array that may be screened for the presence of protecting groups or insufficient elongation with antibodies of the present invention.

Detailed Description of the Preferred Embodiments

1. General Definitions.

"Antibody" as used herein refers to both monoclonal and polyclonal antibodies, refers to antibodies of any immunoglobulin type (including but not limited to IgG and IgM antibodies), and including antibody fragments that retain the hypervariable or binding regions thereof. Antibodies may be of any species of origin, but are typically mammalian (*e.g.*, horse, rat, mouse, rabbit, goat). Antibodies may be bound to or immobilized on solid supports such as nitrocellulose, agarose, glass, organic polymers ("plastics") and the like in accordance with known techniques, and may be labeled with or joined to other detectable groups in accordance with known techniques.

"Binding" as used herein with respect to the selective binding of an antibody to an oligomer has its usual meaning in the art. In general, to obtain useful discrimination in an immunoassay or an affinity purification technique, the antibody should bind to the protected oligomer at an affinity of at least about $k_d = 10^{-6}$, 10^{-7}, or 10^{-8} M, and should bind to the unprotected oligomer at an affinity of not greater than about $k_d = 10^{-2}$, 10^{-3}, or 10^{-4} M.

"Oligomer" as used herein refers to synthetic oligonucleotides and synthetic oligopeptides, including synthetic oligomers in the naturally occurring form such as DNA and RNA, and modified backbone chemistries as discussed below. Oligonucleotides are currently preferred in carrying out the present invention, and the instant invention is primarily explained with reference to oligonucleotides herein. However, the methods and techniques described herein may also be applied to oligopeptides, oligosaccharides, etc. (*i.e.*, any synthetically produced polymer requiring protecting groups for synthesis).

"Nucleotide" as used herein refers to a subunit of an oligonucleotide comprising a pentose, a nitrogenous heterocyclic base (typically bound to the 1 position of the pentose), and a phosphate or phosphoric acid group (typically bound at the 5' position of the pentose) but absent, or considered bound at the 3' position, in the 5' terminal nucleotide of an oligonucleotide. These structures are well known. *See, e.g.*, A. Lehninger, *Biochemistry*, 309-320). "Nucleoside" typically refers to a nucleotide, absent a phosphoric acid or phosphate group.

"Protecting group" as used herein has its conventional meaning in the art and refers to a chemical moiety, group or substituent that is coupled, typically covalently

coupled, to an atom in a molecule prior to a chemical reaction involving that molecule (typically in an organic synthesis), so that the chemical reaction is averted at the atom to which the protecting group is coupled. Typically, the protecting group is then chemically removed from the intermediate molecule for preparation of the final product, although removal techniques may not be entirely successful leading to only partial deprotection of the final product (i.e., the presence of at least one protecting group remaining on that molecule). Protecting groups may be intentionally left on a molecule for purposes of generating or testing an antibody as described herein.

"Deprotection" or "deprotected" as used herein refers to the absence of protecting groups employed during chemical oligonucleotide synthesis from a molecule. Such protecting groups are described below. The presence of such a protecting group may indicate insufficient elongation of the oligonucleotide, when the protecting group is chain terminating. Chemically synthesized oligonucleotides are ideally fully deprotected, but the present invention is employed to detect partial or incomplete deprotection of such oligonucleotides (that is, the presence of at least one protecting group as described below in the oligonucleotide).

"Base" as used herein with respect to oligonucleotides refers to a nitrogenous heterocyclic base which is a derivative of either purine (*e.g.*, adenine, guanine) or pyrimidine (*e.g.*, uracil, thymine, cytosine). Pyrimidine bases are bound to the pentose by the 1 ring nitrogen; Purine bases are bonded to the pentose by the 9 ring nitrogen. Preferred bases are those that contain a free amino group, such as guanine, adenine, and cytosine (the protecting group is then covalently bound to the free amino group by substitution of one, or both, of the hydrogens on the free amino group). However, the present invention may be used with any purine or pyrimidine base, whether standard or modified/rare, that contains a free amino group for protection, or other group requiring protection during synthesis thereof in an oligonucleotide. Examples of standard and modified/rare bases are those found in the nucleosides set forth in **Table 1** below.

Table 1. Standard and modified nucleosides and their standard abbreviations.

abbreviation	base
U	uridine
C	cytidine
A	adenosine
G	guanosine
T	thymidine
?A	unknown modified adenosine
m1A	1-methyladenosine
m2A	2-methyladenosine
i6A	N^6-isopentenyladenosine
ms2i6A	2-methylthio-N^6-isopentenyladenosine
m6A	N^6-methyladenosine
t6A	N^6-threonylcarbamoyladenosine
m6t6A	N^6-methyl-N^6-threonylcarbomoyladenosine
ms2t6A	2-methylthio-N^6-threonylcarbamoyladenosine
Am	2'-O-methyladenosine
I	Inosine
m1I	1-methylinosine
Ar(p)	2'-O-(5-phospho)ribosyladenosine
io6A	N^6-(cis-hydroxyisopentenyl)adenosine
?C	Unknown modified cytidine
s2C	2-thiocytidine
Cm	2'-O-methylcytidine
ac4C	N^4-acetylcytidine
m5C	5-methylcytidine
m3C	3-methylcytidine
k2C	lysidine
f5C	5-formylcytidine
f5Cm	2'-O-methyl-5-formylcytidine
?G	unknown modified guanosine
Gr(p)	2'-O-(5-phospho)ribosylguanosine
m1G	1-methylguanosine
m2G	N^2-methylguanosine
Gm	2'-O-methylguanosine
m22G	N^2N^2-dimethylguanosine
m22Gm	N^2,N^2,2'-O-trimethylguanosine
m7G	7-methylguanosine
fa7d7G	archaeosine
Q	queuosine
manQ	mannosyl-queuosine
galQ	galactosyl-queuosine
Yw	wybutosine
o2yW	peroxywybutosine
?U	unknown modified uridine

abbreviation	base
mnm5U	5-methylaminomethyluridine
s2U	2-thiouridine
Um	2'-O-methyluridine
s4U	4-thiouridine
ncm5U	5-carbamoylmethyluridine
mcm5U	5-methoxycarbonylmethyluridine
mnm5s2U	5-methylaminomethyl-2-thiouridine
mcm5s2U	5-methoxycarbonylmethyl-2-thiouridine
cmo5U	uridine 5-oxyacetic acid
mo5U	5-methoxyuridine
cmnm5U	5-carboxymethylaminomethyluridine
cmnm5s2U	5-carboxymethylaminomethyl-2-thiouridine
acp3U	3-(3-amino-3-carboxypropyl)uridine
mchm5U	5-(carboxyhydroxymethyl)uridinemethyl ester
cmnm5Um	5-carboxymethylaminomethyl-2'-O-methyluridine
ncm5Um	5-carbamoylmethyl-2'-O-methyluridine
D	Dihydrouridine
ψ	pseudouridine
m1ψ	1-methylpseudouridine
ψm	2'-O-methylpseudouridine
m5U	ribosylthymine
m5s2U	5-methyl-2-thiouridine
m5Um	5,2'-O-dimethyluridine

See Sprinzl et al., *Nucleic Acids Res.* **26**, 148 (1998).

Applicants specifically intend that the disclosures of all United States patent references cited herein be incorporated by reference herein in their entirety.

2. Protecting groups.

The particular protecting group will depend upon the oligomer being synthesized and the methodology by which that oligomer is synthesized.

For the synthesis of oligonucleotides, suitable protecting groups include alkyl, aryl, alkylaryl, arylalkyl groups, which may contain one or more hetero atoms such as N, O, or S, and which may be substituted or unsubstituted (*e.g.*, a carbonyl group). Examples of protecting groups include, but are not limited to, the following: acetyl; isobutyryl; 2-(t-butyldiphenyl-silyloxymethyl)benzoyl; naphthaloyl; iso-butyryloxycarbonyl; levulinyl; fluorenylmethoxycarbonyl; 2-nitrothiophenyl; 2,2,2-trichloro-t-butoxycarbonyl, ethoxycarbonyl; benzyloxycarbonyl; p-nitrophenyl-

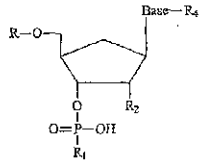
ethyloxycarbonyl; N'N-dimethylformamidine; formyl; benzoyl, toluyl; 2,4-6-trimethylbenzoyl; anisoyl; 2,4-dimethylphenyl; 2,4,6-trimethylphenyl; triphenylthiomethyl; pivoloiloxymethyl; t-butoxycarbonyl; p-nitrophenylethyl; methoxyethoxymethyl; butylthiocarbonyl; 2-methyl-pyridine-5-yl; 2-nitrothiophenyl; 2,4-dinitrothiophenyl; 2-nitro-4-methylthiophenyl; p-nitrophenylsulphonylethyl; 5-chloro-8-hydroxyquinoline; thiophenyl; β-cyanoethyl; phenylethyl; p-nitrophenylethyl; pyridylethyl; 2-N-methylimidazolylphenyl; methyl; allyl; trichloroethyl; dibenzoyl; p-nitrophenylethoxycarbonyl; benzoyl and substituted derivatives thereof; 2(acetoxymethyl) benzoyl; 4,4',4"-tris-(benzyloxy)trityl; 5-methylpyridyno-2-yl; phenylthioethyl; dipehylcarbamoyl; 3,4-dimethoxybenzyl; 3-chlorophenyl; 2-nitrophenyl; 9-phenylxanthen-9-yl; 9-(p-methoxyphenyl)xanthen-9-yl; 9-(p-ocatadecyloxyphenyl)xanthen-9-yl; "bridged" bis-dimethoxytrityl groups; phthaloyl; succinyl; benzensulphonylethoxycarbonyl; 4,4',4"-tris(bevulinyloxy)trityl; p-phenylazophenyloxycarbonyl; o-substituted benzoyl; 4,4'4"-tris-(4,5-dichlorophalimidin)trityl; levelinyl; alkyloxy and aryloxyacetyl; 1,3-benzodithiol-2-yl; tetrahydrofuranyl; [2-(methylthio)phenyl]thiomethyl; 1-(2-chloroethyoxy)ethyl; 1-[(2-fluoro-phenyl]4-methoxy piperidin-4-yl; 4-methoxytetrahydropyran-4-yl; (1-methyl-1-methoxy)ethyl; tetrahydropyranyl; 3-methoxy-1,5-dicarbomethoxypentam-3-yl; 2-nitrobenzyl; benzyl; 4-nitrophenylethyl-sulphonyl; t-butyldimethylsilyl; 4-methoxybenzyl; 3,4-dimethoxybenzyl; 9-p-methoxyphenylthioxanthen-9-yl; compounds of the formula $R_1R_2R_3C$-, wherein R_1, R_2, and R_3 are each independently selected from the group consisting of phenyl, p-monomethoxyphenyl, o-monomethoxphenyl, biphenyl, p-fluoropnehyl, p-chlorophenyl, p-methylphenyl, p-nitrophenyl, etc.

3. *Oligonucleotides.*

Synthetic oligonucleotides that contain protecting groups and may be used to carry out the present invention include both the naturally occurring forms such as DNA and RNA, and those with modified backbone chemistries, such as poly (phosphate derivatives) such as phosphonates, phosphoramides, phosphonamides, phosphites, phosphinamides, *etc.*, poly (sulfur derivatives) *e.g.*, sulfones, sulfonates, sulfites, sulfonamides, sulfenamides, *etc.* It will be noted that antibodies of the

invention may be characterized by their selective binding to particular "reagent" or "benchmark" oligonucleotides, but the same antibodies may also bind to a variety of other oligonucleotides (e.g., longer nucleotides) or other compounds that contain the same protecting group.

For example, an oligonucleotide to which the antibody selectively binds may consist of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I) below:



(I)

wherein:

R is H or a protecting group, such as dimethoxytrityl; subject to the proviso that R is a covalent bond to an adjacent nucleotide when said protected base is not a 5' terminal nucleotide in said oligonucleotide;

R_1 is H or a protecting group such as β-cyanoethyl; subject to the proviso that R_1 is a covalent bond to an adjacent nucleotide when said protected base is not a 3' terminal nucleotide in said oligonucleotide;

R_2 is H or $-OR_3$;

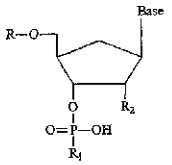
R_3 is H or a protecting group such as *tert*-butyldimethylsilyl;

Base is a purine or pyrimidine base; and

R_4 is a protecting group bonded to an amino group of said base, such as a protecting group is selected from the group consisting of acetyl (Ac), benzoyl (Bz), dimethylformamidine (dmf), isobutyrl (Ibu), phenoxyacetyl (Pac), and isopropyl-phenoxyacetyl (Ipr-pac);

and further subject to the proviso that when one of R, R_1, R_3 and R_4 is a protecting group, then the others of R, R_1, R_3 and R_4 are not protecting groups.

In one particular embodiment of the foregoing, the antibody may be one that selectively binds to an oligonucleotide that consists of from 3 to 20 nucleotides and has a 5' nucleotide, and wherein said 5' nucleotide is a protected nucleotide according to Formula (I):



(I)

wherein:

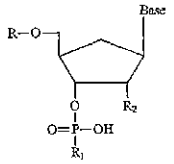
R is a protecting group such as dimethoxytrityl;

R_1 is a covalent bond to an adjacent nucleotide;

R_2 is -H or -OH; and

Base is a purine or pyrimidine base.

In another particular embodiment of the foregoing, the antibody may be one that selectively binds to an oligonucleotide that consists of from 3 to 20 nucleotides and has a 3' nucleotide, and wherein said 3' nucleotide is a protected nucleotide according to Formula (I):



(I)

wherein:

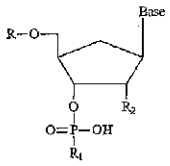
R is a covalent bond to an adjacent nucleotide;

R_1 is a protecting group such as β-cyanoethyl;

R_2 is H or –OH; and

Base is a purine or pyrimidine base.

In another particular embodiment of the foregoing, the antibody may be one that selectively binds to an oligonucleotide that consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I):



(I)

wherein:

R is a covalent bond to an adjacent nucleotide;

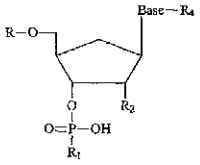
R_1 is a covalent bond to an adjacent nucleotide;

R_2 is $-OR_3$;

R_3 a protecting group such as *tert*-butyldimethylsilyl; and

Base is a purine or pyrimidine base.

In still another particular embodiment of the foregoing, the antibody may be one that selectively binds to an oligonucleotide that consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I):



(I)

wherein:

R is a covalent bond to an adjacent nucleotide;

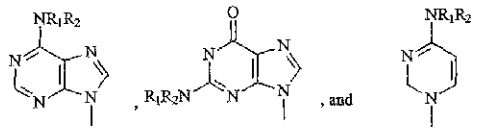
R_1 is a covalent bond to an adjacent nucleotide;

R_2 H or –OH;

Base is a purine or pyrimidine base; and

R_4 is a protecting group bonded to an amino group of said base, such as acetyl, benzoyl, dimethylformamidine, isobutyryl, phenoxyacetyl, and isopropyl-phenoxyacetyl.

Thus, examples of protected bases that may be employed in the structures shown above include, but are not limited to, adenine, guanine, and cytosine, as follows:



wherein R_1 and R_2 are both H in an unprotected base, and either R_1 or R_2 are a protecting group as described above (*e.g.* Pac, Ipr-pac, Ibu, Bz, Ac, dmf) for a protected base. Likewise, modified nucleosides have protecting groups at the modifications that are chemically reactive.

In one embodiment of the invention, the oligonucleotides are peptide nucleic acids, and the protecting groups are those protecting groups employed in the synthesis of peptide nucleic acids, includinb but not limited to those described in U.S. Patent No. 6,133,444.

In still another particular embodiment of the foregoing, the antibody may be one that selectively binds to an oligonucleotide that consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected with a photolabile protecting group, including but not limited to those described in U.S. Patents Nos. 5,744,101 and 5,489,678 (assigned to Affymax).

4. Antibodies.

As noted above, the present invention provides antibodies (*e.g.*, a monoclonal or polyclonal antibody) that specifically bind to a synthetic oligonucleotide having a organic protecting group covalently bound thereto, which antibody does not bind to said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto.

The antibody may be provided immobilized on (or bound to) a solid support in accordance with known techniques, or may be provided in a free, unbound form (*e.g.*, lyophilized, frozen, in an aqueous carrier, etc.). Whether or not an antibody is immobilized will depend upon the particular immunoassay or affinity purification technique in which the antibody is used, and is determined by the known parameters for such techniques. Similarly, the antibody may be bound to or conjugated with suitable detectable groups, such as an enzyme (*e.g.*, horseradish peroxidase), a member of a binding pair such as biotin or avidin, a radioactive group or a fluorescent group such as green fluorescent protein, also in accordance with known techniques, typically depending upon the immunoassay format in which the antibody is used.

5. Immunoassay Methods.

The present invention provides a method for detecting incomplete deprotection of a synthetic oligonucleotide (including aborted sequences that still contain a protecting group) by immunoassay. In general, such an immunoassay comprises the steps of: *(a)* contacting a synthetic oligonucleotide to an antibody as described above, and then *(b)* detecting the presence or absence of binding of said antibody to said oligonucleotide, the presence of binding indicating incomplete deprotection of said synthetic oligonucleotide. Any suitable assay format can be employed, including heterogeneous and homogeneous immunoassays. For example, the immunoassay may be an immunoblot-dot assay, or may be a sandwich assay. The oligonucleotides being tested for deprotection may be in any suitable form, such as in solution or immobilized on a solid support.

In a preferred embodiment, the detection method employs a "dip stick" or the like, in which binding of the antibody to the test oligonucleotide is compared to binding of the antibody to a set of known oligonucleotides, all immobilized on a common solid support. Such an article, as illustrated in **Figure 10**, useful for

determining incomplete deprotection of a synthetic oligonucleotide in an immunoassay, comprises: *(a)* a solid support (*e.g.*, a nitrocellulose strip) **25** having a surface portion, said surface portion having at least two separate discrete regions **26, 27** formed thereon; *(b)* a first oligonucleotide bound to one of said separate discrete regions, said first oligonucleotide having a protecting group bound thereto (*e.g.*, at least one protecting group); and *(c)* a second oligonucleotide bound to another of said separate discrete regions, said second oligonucleotide not having said protecting group bound thereto; wherein the nucleotide sequence of said first and second oligonucleotides are the same. In a preferred embodiment, the article further comprises *(d)* a third oligonucleotide bound to another of said separate discrete regions **28**; said third oligonucleotide also having said protecting group bound to said first oligonucleotide bound thereto; wherein said third oligonucleotide is partially deprotected (*i.e.*, has a number of protecting groups covalently bound thereto which is intermediate between that bound to the first and second oligonucleotide, e.g., at least one, two three or four more protecting groups than the first oligonucleotide, up to at least 10, 20 or more protecting groups than the first oligonucleotide); and wherein the nucleotide sequence of said first, second, and third oligonucleotides are the same. Of course, still more oligonucleotides carrying varying numbers of protecting groups may be included on the substrate in additional separate and discrete locations, if desired. The discrete regions to which the separate oligonucleotides are bound may be in any form, such as dots.

6. Affinity Purification Methods.

In addition to immunoassays, the present invention also provides affinity purification techniques for the separation of fully deprotected oligonucleotides from partially deprotected (including fully protected) oligonucleotides (*e.g.*, both oligonucleotides that have been subjected to a deprotection process to remove the protecting group, and oligonucleotides that have not). Such a procedure typically comprises *(a)* contacting a mixture of protected and fully deprotected synthetic oligonucleotides to antibodies as described above, wherein the protected synthetic oligonucleotides have the organic protecting group for which the antibody is selective covalently bound thereto, so that the protected synthetic oligonucleotides bind to the

antibody; and then separating said antibodies from said fully deprotected oligonucleotides. The antibody may be immobilized on a solid support to facilitate separation. The protected synthetic oligonucleotide may be a partially protected synthetic oligonucleotide, or a fully protected synthetic oligonucleotide that has not undergone deprotection. Any separation format may be used, including but not limited to affinity chromatography.

7. Production of Antibodies.

A method of making an antibody that specifically binds to a synthetic oligonucleotide having a organic protecting group covalently bound thereto, which antibody does not bind to the said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto, comprises the steps of: *(a)* synthesizing the synthetic oligonucleotide on a solid particulate support (and preferably covalently bound thereto, *e.g.*, with a succinyl linker) with the organic protecting group covalently bound to said synthetic oligonucleotide; and then, without removing the oligonucleotide from said solid support; and *(b)* immunizing an animal with the synthetic oligonucleotide bound to the solid support in an amount sufficient to produce the antibody. In addition, a single nucleotide can be bound to the solid particulare support with the organic protecting group bound thereto, and used as described hereinabove.

The synthesis step may be carried out on the solid support in accordance with known techniques. The solid support may be in particulate form prior to synthesis, or may be fragmented into particles after synthesis. In general, the solid supports are beads, which may be completely solid throughout, porous, deformable or hard. The beads will generally be at least 10, 20 or 50 to 250, 500, or 2000 μm in diameter, and are most typically 50 to 250 μm in diameter. Any convenient composition can be used for the solid support, including cellulose, pore-glass, silica gel, polystyrene beads such as polystyrene beads cross-linked with divinylbenzene, grafted copolymer beads such as polyethyleneglycol/polystyrene, polyacrylamide beads, latex beads, dimethylacrylamide beads, composites such as glass particles coated with a hydrophobic polymer such as cross-linked polystyrene or a fluorinated ethylene polymer to which is grafted linear polystyrene, and the like. Where separate discrete

solid supports such as particles or beads are employed, they generally comprise from about 1 to 99 percent by weight of the total reaction mixture.

In a preferred embodiment, the synthesizing step is followed by the step of fragmenting the solid support (*e.g.*, by crushing) prior to the immunizing step. Polyclonal antibodies may be collected from the serum of the animal in accordance with known techniques, or spleen cells may be collected from the animal, a plurality of hybridoma cell lines produced from the spleen cells; and then a particular hybridoma cell line that produces the antibody isolated from the plurality of hybridoma cell lines.

A particular protocol for the production of antiserum/polyclonal antibodies and monoclonal antibodies against protecting groups used in nucleic acid and other synthesis typically involves the following steps: *(a)* preparation of oligonucleotides and others that contain or do not contain protecting groups; *(b)* immunization of animals with those preparations; *(c)* screening of animals to identify those that exhibit antibodies against protecting groups; *(d)* production of monoclonal antibody by classical fusion method; *(e)* optionally, production of scFab, Fab fragments and whole antibody molecules by antibody engineering; and *(f)* evaluation and characterization of monoclonal antibodies against the protecting groups. Each of these steps is discussed in greater detail below.

Synthetic oligonucleotides that contain protecting groups can be synthesized in a variety of ways known to those skilled in the art. For example, protecting groups can be attached to individual nucleotides that are linked to controlled pore glass (CPG) beads. An example is:

CPG bead---dT (only with DMT group).

In the alternative, protecting groups may be attached to oligonucleotide chains that are linked to CPG beads. Examples include:

Pac-dA---Pac-dA---CPG beads with Bz-dC and Ibu-dG;

Ipr-Pac-dG---Ipr-Pac-dG--- CPG beads with Bz-dC and Ibu-dG;

Ac-dC---Ac-dC--- CPG beads with Bz-dC and Ibu-dG;

dmf-G---dmf-G--- CPG beads with Bz-dC and Ibu-dG; and

mixtures of the four oligonucleotides described above.

In another alternative, protecting groups may be attached to oligonucleotide chains that are partially deprotected (the procedure for deprotection will be described bellow). Examples include:

Poly dT20mers (only with DMT group);

Poly dT20mers (only with cyanoethyl groups);

Poly Ibu-dG 20mers (partially deprotected);

Poly Ipr-Pac-dG 20mers (partially deprotected);

Poly Bz-dC 20mers (partially deprotected);

Poly Pac-dA 20mers (partially deprotected); and

Poly Ac-dC 20mers (partially deprotected).

Synthetic oligonucleotides prepared as described herein may be partially deprotected as follows: *(a)* add 30% ammonium hydroxide solution to synthetic polynucleotides, then incubate at room temperature for different time periods (5, 10 and 30 min); *(b)* take the ammonium solution of treated oligomers and add into 1:1 diluted acetic acid pre-cooled at 4° C and according to 1:4 ratio of ammonium to acetic acid; *(c)* keep samples in ice bath for 30 min; *(d)* dry samples with speed-Vac; *(e)* dissolve the dried pellets in water; *(f)* desalt samples with Sephadex G-25 column; *(g)* dry samples with speed-Vac; and *(h)* dissolve the desalted samples in water.

Synthetic oligonucleotides prepared as described herein may be completely deprotected by any suitable technique. One particular technique is as follows: *(a)* add 30% ammonium hydroxide solution to synthetic oligonucleotides, then incubate at 65 ° C for 6 hrs; *(b)* dry samples with speed-Vac; *(c)* dissolve the dried pellets in water; *(d)* desalt samples with Sephadex G-25 column; and *(e)* dry samples with speed-Vac; *(f)* redissolve desalted samples in water.

Partially and completely deprotected oligonucleotides may be characterized for further use or to verify procedures by any suitable means, including but not limited to gel electrophoresis, urea-acrylamide gel electrophoresis, 5'end labeling with T4 polynucleoide kinase, HPLC analysis, mass spectrometry, etc.

Suitable animals can be immunized with the oligonucleotides described above by parenteral injection of the oligonucleotide in a suitable carrier, such as sterile saline solution. Injection may be by any suitable route, including but not limited to

subcutaneous, intraperitoneal, intravenous, intraarterial, intramuscular, etc. Suitable animals are typically mammals, including mice, rabbits, rats, etc.

In a particular embodiment, for the production of monoclonal antibodies, young female BALB/c mice are used, and the time course of injection of the antigen material is:

first day	initial injection
14th day	first boosting
28th day	second boosting
4 day before fusion	final boosting

Additional injections may be employed if desired. The antigen amount may be 50 μg or 100 μg of oligonucleotides unprotected (for control antibody) or protected, for each mouse per time. When, as preferred, beads or other solid support used as the support for oligonucleotide synthesis are injected into the animal, the beads or particles are suspended in water, then injected into mice. If a nucleotide solution is used, then the solution is mixed with complete or incomplete Freund's adjuvant and injected into mice.

Polyclonal antibodies can be harvested from animals immunized or innoculated as described above in accordance with known techniques, or spleen cells harvested from the animals, hybridoma cell lines produced from the spleen cells, and the hybridoma cell lines screened for the production of desired antibodies, also in accordance with known techniques.

Oligonucleotides that contain or do not contain biotin molecules at 3' or 5' ends (for ELISA assay as described below) may be synthesized in accordance with standard techniques. Examples are:

Poly Ibu-dG 20 mers (with or without biotin);

Poly Ibu-dA 20 mers (with or without biotin);

Poly Ibu-dC 20 mers (with or without biotin);

Poly Ipr-Pac-dG 20 mers (with or without biotin);

Poly Bz-dC 20 mers (with or without biotin);

Poly Bz-dA 20 mers (with or without biotin);

Poly dT 20 mers (with or without biotin);

Poly Pac-dA 20 mers (with or without biotin);

Poly Ac-dC 20 mers (with or without biotin); and

Poly dmf-G 20 mers (with or without biotin).

Antibodies produced as described above may be characterized by any suitable technique to determine the binding properties thereof, including but not limited to Western blot and immunodot-blot.

In addition to the use of polyclonal and monoclonal antibodies, the present invention contemplates the production of antibodies by recombinant DNA, or "antibody engineering" techniques. For example, mRNA isolated from hybridoma cells may be used to construct a cDNA library and the sequence encoding whole antibody or antibody fragments (*e.g.*, scFab or Fab fragments) isolated and inserted into suitable expression vector, and the expression vector inserted into a host cell in which the isolated cDNA encoding the antibody is expressed.

Monoclonal Fab fragments may be produced in *Escherichia coli* by recombinant techniques known to those skilled in the art. See, e.g., W. Huse, *Science* 246, 1275-81 (1989).

8. Screening of Antibodies.

Screening sera and hybridoma cell culture media for protecting group specific antibodies may be carried out as follows:

A. Sera

1. Pre-immune (prior to immunization) sera are collected by standard means from the mice to be inoculated with protecting group conjugated to a solid support (directly or through an oligomer).

2. Post-innoculation sera are also collected.

3. An ELISA assay is performed in which the specific protecting group remains on a biotinylated oligonucleotide conjugated to the microtiter plate. Other microtiter plate wells contain control oligomers that have no protecting groups, or oligonucleotides with other protecting groups. The secondary antibody is a goat anti-mouse IgG with a conjugated phosphotase for visualization of antibody

4. Those mice that have positive activity against the specific protecting group are boosted and sacrificed for the production of hybridomas.

B. Hybridoma cell culture media

1. Approximately 1000 cultures are generated from each spleen hybrid cell production.

2. Cultures are grown in microtiter plate wells, 96 well plates.

3. Culture medium is removed from each well and used in ELISA assays as described above in which each of the ~1000 microtiter plate wells contain the protected oligonucleotide conjugated to the plate.

4. Those cultures producing antibody that has positive activity are transferred to larger culture wells, 24 well microtiter plates.

5. Culture media from the larger cultures are re-tested for activity against the protecting group and are also assayed for specificity; ie. controls of no protecting group and of other protecting groups.

6. Those cultures that are positive are cloned out (diluted), re-tested and cloned out again to the point that each final culture must be the result of one cell; ie. mono-culture. Media from these final cultures are thoroughly assessed for specificity and affinity. Specificity and affinity are assessed using a dot-blot assay.

C. Dot-Blot assays in lieu of ELISA assays

1. Antibodies against some protecting groups are not tractable to being tested in the microtiter plate well environment and must be tested using a dot-blot assay. One example is the 5'-terminal protecting group, dimethyl-trityl (DMT).

2. The Dot-blot assay on a nitrocellulose membrane is accomplished as described elsewhere in the application for most purposes. However, this is not possible in assessing antibody production by ~1000 microtiter well cultures with little media available. Thus, a novel adaptation has been developed.

a) The protected oligonucleotide is attached in dots to the nitrocellulose using UV-crosslinking. With DMT, the presence of the 5'-DMT on the membrane is confirmed by treatment of a dot with mild acid -- the dot turns yellow-orange. The presence of the 3'-biotin can be confirmed with a commercial avidin stain.

b) The membrane is blocked (see dot-blot assay).

b) The dry membrane dots are carefully marked (pencil) and "punched" out of the membrane.

c) Individual dots are added to the cell culture media in individual micortiter plate wells and incubated.

d) The individual dots are removed and passed on through the washing, secondary antibody, phosphotase reaction and color development using microtiter plate wells with the appropriate reagents.

e) Those dots that are positive are related back to the original microtiter plate well cultures from which the small amount of culture media was obtained.

f) Further culturing and cloning is accomplished as described in B.

9. Testing of Microarrays.

The present invention may be used to test or screen oligonucleotides that are immobilized on a solid support such as a microarray for insufficient deprotection or elongation of the oligonucleotides synthesized thereon.

Solid supports used to carry out the present invention are typically discrete solid supports. Discrete solid supports may be physically separate from one another, or may be discrete regions on a surface portion of a unitary substrate. Such "chip-type" or "pin-type" solid supports are known. *See, e.g.*, U.S. Patent No. 5,143,854 to Pirrung; U.S. Patent No. 5,288,514 to Ellman (pin-based support); U.S. Patent No. 5,510,270 to Fodor et al. (chip-based support). Additional non-limiting examples of oligonucleotide arrays which may be used to carry out the present invention, and methods of making the same, include but are not limited to those described in U.S. Patents Nos. 5,631,734; 5,599,695; 5,593,839; 5,578,832; 5,510,270; 5,571,639; 6,056,926; 5,445,934; and 5,703,223. Such devices may be used as described therein to carry out the instant invention.

The solid support or substrate from which the array is formed may be comprised of any suitable material, including silicon. The oligonucleotides may be polymerized or grown in situ from monomers (or individual nucleotides) *in situ* on the microarray (in which case none of the currently available techniques for detecting protecting groups would be useful for detecting incomplete deprotection or elongation of the oligonucleotides on the array, as one cannot pass the solid support through an analytical device) or the oligonucleotides may be polymerized separately and then linked to the appropriate regions of the solid support. The array may include any number of different oligonucleotides in different separate and discrete regions

thereon, examples including arrays of at least 1,000, at least 2,000, at least 10,000, or at least 20,000 different oligonucleotides in different separate and discrete regions.

In general, a method of screening an oligonucleotide array for insufficient deprotection or insufficient elongation of oligonucleotides therein comprises the steps of:

(a) providing an oligonucleotide array as described above;

(b) providing an antibody as described above (that is, an antibody that specifically binds to a synthetic oligonucleotide having an organic protecting group covalently bound thereto, which antibody does not bind to said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto). Preferably the antibody is one that specifically binds to an oligonucleotide having a protecting group, where the protecting group was employed in the course of the organic synthesis of oligonucleotides carried by that array. Then;

(c) contacting said oligonucleotide array to said antibody to thereby detect the presence of insufficient deprotection or insufficient elongation of oligonucleotides therein. Such detection, which may be qualitative or quantitative, may be carried out by any suitable immunoassay technique as described above.

In the method, steps *(b)* to *(c)* may be repeated at least once, with a different antibody on each repetition, so that a plurality of different protecting groups which may be present on oligonucleotides in the array may be detected.

Preferably, once insufficient deprotection (the presence of protecting groups) in oligonucleotides in one or more (e.g., plurality) of the separate and discrete regions is detected, the method further comprises generating a record or indicia recording the presence of insufficient deprotection or insufficient elongation of oligonucleotides in the least one separate and discrete location (or plurality of separate and discrete locations) on the array. The indicia may be a qualitative or quantitative indicia of insufficient deprotection (including insufficient elongation).

The foregoing methods provide a correctable oligonucleotide array as illustrated in **Figure 11**. The array comprises, in combination:

(a) a substrate **30** having a plurality of different oligonucleotides immobilized thereon, with the different oligonucleotides immobilized in different separate and discrete locations **31** on said substrate; and

(b) a plurality of indicia associated with said array, these indicia recording the presence of insufficient deprotection or insufficient elongation of a plurality of different oligonucleotides, said different oligonucleotides located in separate and discrete locations on said array. These indicia may be printed in a region of the array **32** by a technique such a microlithography, printed on conventional medium such as paper and shipped with the array, stored in a memory or memory device connected to or formed on the array chip (which may be incorporated at location 32), provided in a separate data or computer file which may be provided on a computer-readable medium such as a floppy diskette or CD-ROM, stored on a web site on the world wide web for downloading by the end user of the array, etc. When the indicia are provided in a separate data file, the array preferably further includes an identifier such as a code number formed on, connected to or associated with the array (e.g., printed on a package containing the array, or on an information sheet packaged with the array, and/or printed directly on the array). The indentifer may then be associated with the separate indicia (e.g., printed on a data sheet, used as a pass-word, file identifier and/or access code for the computer file, etc.) to insure the correct indicia containing the record of insufficient deprotection and/or elongation are ultimately associated with the array by the ultimate end user of the array.

A data device or memory device connected to the array may be carried out in accordance with known techniques, as described in U.S. Patents Nos. 5,925,562; 6,017,496; 5,751,629; and 5,741,462, and such devices used as described therein to carry out the instant invention.

The end user of the array may utilize the indicia described above to compensate for insufficient deprotection or insufficient elongation of oligonucleotides on said array in a method comprising:

(a) providing a substrate as described above.

(b) providing at least one, or a plurality of, indicia associated with said array as described above.

(c) providing a test compound. The test compound may be a member of a library of test compounds, and may be any suitable compound such as a protein, peptide or oligonucleotide (e.g., a DNA or RNA, such as mRNA); and then

(d) detecting the binding of said test compound to at least one of said plurality of different oligonucleotides (e.g., by contacting the test compound to the array); and then

(d) detecting determining the degree of binding (including simply the presence or absence of binding) of the test compound to one or more oligonucleotides on the array from *(i)* said detected binding and *(ii)* said indicia recording the presence of insufficient deprotection or insufficient elongation. Thus, insufficient deprotection or insufficient elongation of oligonucleotides in one or more locations in the array may be compensated for during the determining step. Such compensation may be achieved by any means, including ignoring particular separate and discrete regions on the array (e.g., in favor of other separate and discrete regions of the array that contain the same oligonucleotide). In another example, if one or more locations contain insufficient deprotection or elongation such that binding to those locations is reduced, the binding data derived from an experiment with that array can be adjusted upwards for those locations to indicate greater binding than that which would otherwise be indicated without the control made possible by the recorded indicia. The detecting or determining step may be carried out by any suitable means, such as generating a color indication of degree of binding, generating a numeric indication of degree of binding, generating a graphic or other symbolic indication of degree of binding, etc. The degree of binding may be an indication of binding is binding affinity, binding amount, or both binding affinity and binding amount, but is typically an indication of the amount of test compound that binds to a particular separate and discrete region of the array.

The present invention is explained in greater detail in the following non-limiting Examples.

EXAMPLE 1

Synthesis of Oligonucleotides

Synthesis was performed on an ABI DNA/RNA Synthesizer, Model 394 (PE Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404) according to manufactories protocol. Slightly modified 1 micromolar scale cycle was used during

synthesis (see manufacturer's instructions). The primary starting materials (and suppliers/manufacturers in parentheses) were as follows:

Activator (0.45 M tetrazole in acetonitrile), CAP A (acetic anhydride/ tetrahydrofuran/ 2,6 lutidine), CAP B (N-methyl imidazole/tetrahydrofuran) and oxidizer (0.02 M iodine/pyridine/THF/H2O) (Prime Synthesis)

Pac-dA (5'-dimethoxytrityl-N-phenoxyacetyl-2'-deoxyAdenosine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research)

Ipr-Pac-dG (5'-dimethoxytrityl-N-p-isopropyl-phenoxyacetyl-2'-Guanosine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research)

Ac-dC (5'-dimethoxytrityl-acetyl-2'-deoxycytidine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research)

dmf-G (5'-dimethoxytrityl-dimethylformamidine-Guanosine,2'-O-TBDMS-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research)

Bz-dC---CPG beads (5'-dimethoxytrityl-N-benzoyl-2'-deoxycytidine, 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite-succinyl linker-beads (3000 Ang) (CPG Inc.)

Ibu-dG---CPG beads (5'-dimethoxytrityl-N-isobutyl—2'-deoxycytidine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite-succinyl linker-beads (3000 Ang) (CPG Inc.)

The following compounds were synthesized, the compounds being linked to beads as shown:

Pac-dA---Pac-dA---Bz-dC---succinyl linker---Beads

Pac-dA---Pac-dA---Ibu-dG—succinyl linker---Beads

Ipr-Pac-dG---Ipr-Pac-dG---Bz-dC---succinyl linker---Beads

Ipr-Pac-dG---Ipr-Pac-dG---Ibu-dG---succinyl linker---Beads

Ac-dC---Ac-dC---Bz-dC---succinyl linker---Beads

Ac-dC---Ac-dC---Ibu-dG---succinyl linker---Beads

dmf-G---dmf-G--- Bz-dC---succinyl linker---Beads

dmf-G---dmf-G--- Ibu-dG---succinyl linker----Beads

The foregoing compounds were administered directly to animals as an immunogen, without separating the oligonucleotide from the solid support, for the production of antibodies, as further described in Example 2 below.

EXAMPLE 2

Innoculation of Animals

Female BALB/c mice of eight to twelve weeks old were purchased from Charles River, Raleigh, North Carolina, USA. The mice were housed in cases with filter caps.

After oligonucleotide chain synthesis was completed as described in Example 1, the beads with nucleotides were gently crushed by hand-pressuring the glass plates, between which beads were positioned.

5 μM of each eight oligonucleotides mentioned above were mixed in 4 ml PBS (150 mM sodium chloride in 100 mM phosphate buffer, pH 7.2).

The mixture was thoroughly vortexed suspending the crushed beads. 150 μL of the vortexed mixture was taken and added into 300 μL of PBS in a syringe. Just before injection, the solution containing beads was mixed again by shaking the syringe to suspend the broken beads. Then 150 μL or 300 μL of well-mixed solution was injected into mouse peritoneal cavity. This procedure was used for the first injection and the following boosts.

Injection time schedule:

Injection	Date (day)
first	0
second	14th
third	28th
4th	42nd
5th	56th
6th	70th
7th	84th
8th	98th
9th	112th
10th	138th
11th (final, 4 day before fusion)	142nd

Four days after the final injection, spleen cells are harvested from the animals and fused with myeloma cells (P3x.63.Ag8.653) in accordance with known techniques to produce hybridoma cell lines, which are then screened to determine the

binding characteristics as described below to isolate particular cell lines that produce the desired antibody of the invention.

EXAMPLE 3

Immunodot-Blot Assay for Antibody Characterization

The Immunodot-blot assay involves UV cross linking of oligonucleotides onto membrane paper, and is directly applicable to a test kit for detection, identification and quantifying the protecting groups on product oligomers. This procedure may be carried out as follows: *(a)* wet membrane paper with TBS (10 mM Tris, pH 7.2; 150 mM NaCl); *(b)* blot oligonucleotides to be tested onto membrane paper under vacuum; *(c)* UV cross link nucleotide onto membrane paper; *(d)* block membrane paper with 1% casein-TBST (TBS plus Tween 20, 0.1% by volume) at room temperature for 2 hr or 4° C overnight; *(e)* wash membrane with TBST 3 times, each for 15 min; *(f)* form antigen-antibody complex by incubation of plate with sample be tested (diluted in 1% casein-TBST) at room temperature for 1 hr; *(g)* wash as above; *(h)* react with second antibody conjugate (diluted in 1% casein-TBST) at room temperature for 1 hr; *(i)* wash as above; *(j)* develop color reaction by incubation of membrane with substrate solution.

EXAMPLE 4

Dot-Blot Assay of Monoclonal Antibody 1 H11

Monoclonal antibody 1 H11, produced as described in Example 2 above, was characterized by a dot-blot assay as described in Example 3 above. Results are shown as a bar graph in **Figure 1**. In Figure 1, lanes (or columns) 1 and 2 represent oligoPac-dA20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes at 4° C, respectively. Columns 3 and 4 represent oligoBz-dC20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 5 and 6 represent oligoAc-dC20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 7 and 8 represent oligoIpr-Pac-dG20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 9 and 10 represent oligoIbu-dG20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 11, 12 and 13 represent oligodT20mers, completely deprotected, with DMT group only,

and with cyanoethyl group only, respectively. Antibody activity is given as optical density (479 nm) from ELISA (Example 7 below), and the positive or negative result of the dot blot assay is given in the open or filled circle appearing over each column in the bar graph. Note the activity of monoclonal antibody 1 H11 in selectively binding to the oligoIbu-dG20mer in column 10.

EXAMPLE 5

Dot-Blot Assay of Monoclonal Antibody 7 H3

Monoclonal antibody 7 H3, produced as described in Example 2 above, was characterized by a dot-blot assay as described in Example 3 above. Results are shown as a bar graph in **Figure 1.** In Figure 1, lanes (or columns) 1 and 2 represent oligoPac-dA20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes at 4° C, respectively. Columns 3 and 4 represent oligoBz-dC20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 5 and 6 represent oligoAc-dC20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 7 and 8 represent oligoIpr-Pac-dG20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 9 and 10 represent oligoIbu-dC20mers treated with NH_4OH for 6 hours at 65° C and 15 minutes, respectively. Columns 11, 12 and 13 represent oligodT20mers, completely deprotected, with DMT group only, and with cyanoethyl group only, respectively. Antibody activity is given as optical density as described above, and the positive or negative result of the dot blot assay is given in the open or filled circle appearing over each column in the bar graph. Note the activity of monoclonal antibody 1 H11 in selectively binding to the oligoBz-dC20mer in column 4.

EXAMPLE 6

Western Blot Assay for Antibody Characterization

The Western blot assay involves low voltage transfer of oligonucleotides from gel to membrane paper and UV cross linking of oligonucleotides onto the membrane. This assay may be carried out as follows: *(a)* cast 15% non-denaturing gel containing 10 mM MgCl; *(b)* load oligonucleotides (oligomers) into the wells of the gel; *(c)* run gel at 200 voltage in ice bath; *(d)* transfer oligonucleotides from gel to membrane

paper at 25 voltage for 25 min in ice bath; *(e)* UV cross link polynucleotides on membrane; *(f)* block membrane paper with 1% casein-TBST at room temperature for 2 hr or 4° C overnight; *(g)* wash membrane with TBST 3 times, each for 15 min; *(h)* incubate samples be tested (diluted in 1% casein-TBST) at room temperature for 1 hr; *(i)* wash as above; *(j)* incubate membrane with second antibody conjugate (diluted in 1% casein-TBST) at room temperature for 1 hr; *(k)* wash as above; and *(l)* color-develop by incubation of membrane with substrate solution.

EXAMPLE 7

Detection of antibody using Biotinylated Polynucleotides as Antigen and an ELISA involving Streptavidin-Biotin System

An enzyme-linked immunosorbent assay (ELISA) for the detection of the antibody is carried out as follows: *(a)* pre-screen microtiter plate that is pre-coated with streptavidin; *(b)* coat the plate with a preparation of biotinylated oligonucleotide or other materials to be tested (at 5 μg/ml in PBS)(PBS: 150 mM NaCl, 10 mM Phosphate buffer, pH 7.4), then incubate at room temperature for 2 hrs; *(c)* wash 3 times with 0.1% Tween in PBS (PBST), each for 15 min; *(d)* block with 1% casein in PBST at room temperature for hrs or 4° C overnight; *(e)* wash as above; *(f)* form antigen-antibody complex by incubation of plate with antibody (or antibodies) at room temperature for 1 hr; *(g)* wash as above; *(h)* react with second antibody-peroxidase conjugate (in 1% casein-PBST) at room temperature for 1 hr; *(i)* wash as above; *(j)* develop color reaction by adding tetramethylbenzidine (TMB) solution (TMB solution: 42 mM TMB, 0.004% H_2O_2, 0.1 M acetate buffer, pH 5.6) and incubating at room temperature for 15 min, then stop the reaction with 2 M H_2SO_4; and *(k)* read absorption value at 469 nm.

EXAMPLE 8

ELISA and Dot-Blot Assay of Monoclonal Antibody Against Benzoyl, Isobutryl, and Isopropylphenoxyacetiyl

Monoclonal antibodies (mAb) against protecting groups benzoyl (Bz), isobutryl (ibu), and isopropylphenoxyacetiyl (ipr-Pac), produced as described in Example 2 above, were characterized by a standard ELISA assay and a dot-blot assay

as described in Example 3 above. An ELISA assay developed with biotinylated nucleic acids of 20 residues each attached to a 96-well microtiter plate demonstrated the specificity of the antibodies for their respective antigens. **Figure 3A, Figure 4A,** and **Figure 5A** show results for monoclonal antibodies against Bz, ibu, and ipr-Pac, respectively. The figures show completely deprotected (<1% Bz remaining) homopolymers of dC residues, designated oligo dC(Bz), ie. originally protected with Bz (lane 1, open bar), protected (>97% Bz remaining) oligo Bz-dC (lane 2, shaded bar), completely (< 1% ipr-Pac remaining) deprotected oligo dG(ipr-Pac) (lane 3), protected (>76% ipr-Pac) oligo ipr-PacdG (lane 4), completely (< 1% ibu remaining) deprotected oligo dG(ibu) (lane 5), protected (>91% ibu remaining) oligo ibu-dG (lane 6), and completely deprotected oligo dT (lane 7). The dT polymer had but one protecting group, dimethyltrityl (DMT) that was removed from the 5'OH of the 5'-terminal residue with mild acid. Finally, lane 8 of shows oligo dT with DMT remaining.

Dot-Blot assays of anti-Bz mAb, anti-ibu mAb, and anti-ipr-Pac mAb activities were performed in which the 20mer DNAs were linked to nitrocellulose membrane by UV. The amounts of 20mer DNA applied to the membrane are shown to the right of **Figure 3B, Figure 4B,** and **Figure 5B** and demonstrate the level of sensitivity of the assay. The DNAs used to test anti-Bz mAb were those described for the ELISA plus deprotected oligo dA(Bz), protected oligo Bz-dA, oligo dC(ibu), oligo ibu-dC, oligo dA(ibu) and oligo ibu-dA. **Figure 3B** shows that the anti-Bz mAb recognized the protecting group on dA and dC. The DNAs used to test anti-ibu mAB were those described for the ELISA plus protected oligo ibu-dA, deprotected oligo dA(ibu), oligo ibu-dC, oligo dC(ibu) and all are noted at the top of the dot-blot. **Figure 4B** shows that the anti-ibu mAb recognized ibu on dG, the most common use of the protecting group, but also on dA. The DNAs used to test anti-ipr-Pac mAb were those described for the ELISA plus protected oligo ibu-dA, deprotected oligo dA(ibu), oligo ibu-dC, oligo dC(ibu), oligo Bz-dA, oligo dA(Bz) and all are noted at the top of the dot-blot. **Figure 5B** shows that the anti-ipr-Pac mAb recognized ipr-Pac on dG, the most common use of the protecting group, but also on dA and dC. The mAb also recognized the ibu protecting group (ibu-dG, ibu-dA and ibu-dC). This cross-reactivity indicates that the antibody was highly selective in its identification of

a chemistry common to both ipr-Pac and ibu, possibly $CH(CH_3)_2$. Thus the anti-ibu and anti-iprPac mAbs could be used in combination to identify the protecting group remaining on an oligo.

Greater amounts of DNA were tested in a dot blot assay of anti-ibu mAb (**Figure 4C**). The results of this experiment demonstrated that the ibu protecting group was recognized by the mAb no matter which nucleobase was protected.

Figure 3C, **Figure 4D**, and **Figure 5C** demonstrate that partially deprotected oligomers can be re-treated to remove the remaining protecting groups, and re-tested with mAb. **Figure 3C** shows that anti-Bz mAb recognized re-deprotected oligomer oligo Bz-dC (center column). Likewise, **Figure 4D** shows that anti-ibu mAb recognized re-deprotected oligomer oligo ibu-dG (center column) and **Figure 5C** shows that anti-ipr-Pac mAb recognized re-deprotected oligomers oligo ipr-Pac-dG and oligo ibu-dG (columns second from left and forth from left, respectively). Thus, this approach is applicable to quality control without having to discard expensive nucleic acid samples.

An RNA standard with protecting groups Bz, ibu and ipr-Pac was synthesized and assayed for identification of the protecting groups with the mAb against Bz (**Figure 3D**), ibu (**Figure 4E**), and ipr-Pac (**Figure 5D**). Dot-blot assays clearly show that the monoclonal antibodies do not differentiate RNA from DNA. Although there was a higher background signal with RNA than with DNA, there was a significant distinction between RNA with and without protecting groups, especially at the lower amounts of RNA. The amount of RNA on the membrane was estimated from the optical absorbance of the sample.

EXAMPLE 9

mAb Dot-Blot Assay of Protecting Groups vs HPLC

Dot-blot detection of Bz groups remaining on a standardized 20mer oligo dC molecule were performed as described in Example 3. Completely deprotected and the untreated oligo dC 20mers were analyzed for the Bz protecting group using a totally independent and different quantification method. The two oligomers were hydrolyzed to the constituent nucleosides and then their nucleoside composition identified and quantified using a recognized high performance liquid chromatography (HPLC)

method with concentrated samples. Because of the lack of sensitivity, HPLC detection required 50-100 fold the amounts of Bz-dC used in the mAb assays (see **Figure 7**). **Figure 6A** shows the result of anti-Bz mAb tested against nmole amounts of Bz groups on ptotected oligo Bz-dC (right column) and the same nmole amounts of Bz- on Bz-dC (left column). Each amount of Bz-dC oligo was diluted with completely deprotected dC oligo of the same length (20mer) to demonstrate the sensitivity of the mAb detection even in the presence of 2500-fold dC (ie. 0.04%). The mAb assay demonstrated that the mAb could detect the Bz group on DNA even in the presence of a 2500-fold excess of dC in DNA.

The dot-blot shown in **Figure 6A** was subjected to densitometry to quantitate the mAb response. After background subtraction, the remaining density was plotted as a function of Bz groups in oligo Bz-dC determened by HPLC (**Figure 6B**). The data indicated that the high sensitivity of the anti Bz mAb detection was linear in 0.1-1.0 nmol range.

Next, it was determined whether the mAb response could be enhanced with an increase in the amount of DNA on the dot-blot membrane. The amount of Bz was determined by standard HPLC methods. This experiment showed that detection of the Bz protecting group in a mixture of the protected sample with the deprotected sample at a ratio of 1/2500 could be enhanced by increasing the amount of DNA on the membrane, though the ratio was maintained (**Figure 6C**).

Finally, experiments were conducted to show a direct comparison of the mAb and HPLC detection of Bz. Anti-Bz mAb was utilized in a dot-blot assay to detect Bz on dC in the oligo Bz-dC (20mer). The density response of the Bz group detected Bz by the mAb assay and quantified by densitometry was plotted against the amount of Bz in the DNA on each dot (**Figure 7A**). The amount of Bz in the DNA was calibrated by digestion of a large amount of DNA and analysis by HPLC identification and quantification of the Bz-dC mononucleoside. For HPLC experiments, three samples of Bz-dC oligo were hydrolyzed and analyzed for composition by HPLC. The response of the UV-diode array detector was plotted against the amount of Bz in the samples (**Figure 7B**). The sample amounts were determined by comparison to samples "spiked" with known amounts of Bz-dC. The amounts of Bz-dC added to samples as spikes were from a weighed stock of Bz-dC.

Thus, the HPLC response was calibrated with known amounts of Bz-dC. The results of these experiments show that the detection of Bz by anti-Bz mAb was within the pmole range whereas HPLC detection of Bz was limited to the nmole range.

EXAMPLE 10

Detection of Remaining Protecting Groups in Commercial Samples

A blind study was conducted to demonstrate the detection of remaining protecting groups in commercial samples by mAb. The purpose of the this experiment was to determine if protecting groups could be detected and identified with mAb technology in presumably completely deprotected samples that had been treated as commonly accomplished in the oligo synthesis industry. The nature of the protecting groups used by eight selected companies was not known, thus the experiment was a blind study. Two 20mer oligos (oligo dA-dC and oligo dG-dT) from each of the eight companies were ordered to be synthesized and deprotected, and salt removed under as identical conditions as possible. The oligos were shipped by express mail, as is often the case, and then subjected to mAb analysis by dot blot. The dA-dC oligo from one company (#6), and possibly a second (#2), had remaining Bz protecting groups as determined by anti-Bz mAb testing (**Figure 8A**). The dG-dT oligos from two companies (#2 and #6) had ipr-Pac protecting groups remaining as determined by anti-ipr-Pac mAb (**Figure 8B**). The remaining protecting groups in the commercial samples were confirmed by increasing amounts of sample and further deprotection and re-analyses. The oligo dA-dC samples from companies #2 and #6 were tested in higher amounts to confirm the presence of the Bz protecting group. In addition, the samples were treated to remove the remaining protecting groups using a standard protocol. The re-analysis after further deprotection indicated that the groups were now removed (**Figure 8C**). This also demonstrates that expensive nucleic acid samples can be re-treated to remove protecting groups and that they need not be discarded. The oligo dG-dT samples were re-treated to remove remaining protecting groups and re-analyzed with anti-ipr-Pac mAb with the result that the ipr-Pac group could be removed without sacrificing the DNA (**Figure 8D**).

EXAMPLE 11

Polyclonal Antibody Against Dimethyltrityl

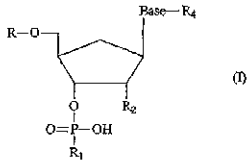
Production and analyses of polycolonal antibody against the 5' terminal protecting group, dimethyltrityl (DMT) were as described in Example 2. Four mice were inoculated with DMT and sera were drawn from the mice after some weeks of boosting with antigen. DMT [DMT—OH], three DMT at the 5'-end of the deoxynucleotide trimer $d(T)_3$ [$(DMT)_3$-$d(T)_3$], three DMT at the 5'-end of the deoxynucleotide 20mer $d(T)_3$ with 3'-biotin [$(DMT)_3$-$d(T)_{20}$-biotin], one DMT at the 5'-end of the deoxynucleotide 20mer $d(T)_{20}$ with 3'-biotin [DMT-$d(T)_{20}$-biotin], the dT 20mer with 3'-biotin [$d(T)_{20}$-biotin], one DMT with biotin [DMT-biotin] and tris-borate saline control were applied to a nitrocellulose membrane that was then assayed with mouse sera (inoculated mice #1-4 and a control serum, normal) to assess anti-DMT antibody, mild acid to reveal presence of the DMT (TBS), and avidin to reveal the presence of biotin (**Figure 9**). Sera from mice #2 and #4 recognized DMT [as $(DMT)_3$-$d(T)_3$], whereas mice #1, #3, and the normal mouse did not. Mild acid revealed the presence of DMT as a yellow color (not visible in figure) and avidin revealed the presence of biotin.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. An antibody that specifically binds to a synthetic oligonucleotide having a organic protecting group covalently bound thereto, which antibody does not bind to said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto.

2. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I):



(I)

wherein:

R is H or a protecting group;

subject to the proviso that R is a covalent bond to an adjacent nucleotide when said protected base is not a 5' terminal nucleotide in said oligonucleotide;

R_1 is H or a protecting group;

subject to the proviso that R_1 is a covalent bond to an adjacent nucleotide when said protected base is not a 3' terminal nucleotide in said oligonucleotide;

R_2 is H or $-OR_3$;

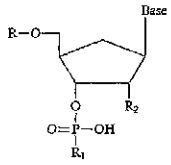
R_3 is H or a protecting group;

Base is a purine or pyrimidine base;

R_4 is a protecting group bonded to an amino group of said base;

and further subject to the proviso that when one of R, R_1, R_3 and R_4 is a protecting group, then the others of R, R_1, R_3 and R_4 are not protecting groups.

3. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides and has a 5' nucleotide, and wherein said 5' nucleotide is a protected nucleotide according to Formula (I):



(I)

wherein:

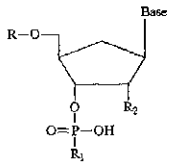
R is a protecting group;

R_1 is a covalent bond to an adjacent nucleotide;

R_2 is -H or –OH; and

Base is a purine or pyrimidine base.

4. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides and has a 3' nucleotide, and wherein said 3' nucleotide is a protected nucleotide according to Formula (I):



(I)

wherein:

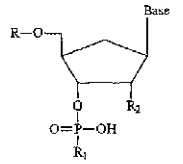
R is a covalent bond to an adjacent nucleotide;

R_1 is a protecting group;

R_2 is H or –OH; and

Base is a purine or pyrimidine base.

5. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I):



(I)

wherein:

R is a covalent bond to an adjacent nucleotide;

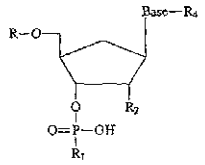
R_1 is a covalent bond to an adjacent nucleotide;

R_2 is $-OR_3$;

R_3 a protecting group; and

Base is a purine or pyrimidine base.

6. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected nucleotide according to Formula (I):



(I)

wherein:

R is a covalent bond to an adjacent nucleotide;

R_1 is a covalent bond to an adjacent nucleotide;

R_2 is H or –OH;

Base is a purine or pyrimidine base; and

R_4 is a protecting group bonded to an amino group of said base.

7. An antibody according to claim 1, wherein said oligonucleotide consists of from 3 to 20 nucleotides, and wherein one of said nucleotides is a protected with a photolabile protecting group.

8. An antibody according to claim 1, which antibody is a polyclonal antibody.

9. An antibody according to claim 1, which antibody is a monoclonal antibody.

10. An antibody according to claim 1 immobilized on a solid support.

11. A cell that expresses an antibody according to claim 9.

12. A cell according to claim 11, which cell is a hybridoma.

13. A cell according to claim 11, which cell contains and expresses a heterologous nucleic acid encoding said antibody.

14. A method for detecting incomplete deprotection of a synthetic oligonucleotide by immunoassay, said immunoassay comprising the steps of:

contacting a synthetic oligonucleotide to an antibody according to claim 1; and then

detecting the presence or absence of binding of said antibody to said oligonucleotide, the presence of binding indicating incomplete deprotection of said synthetic oligonucleotide.

15. A method according to claim 14, wherein said immunoassay is a heterogeneous immunoassay.

16. A method according to claim 14, wherein said immunoassay is a homogeneous immunoassay.

17. A method according to claim 14, wherein said immunoassay is a sandwich assay.

18. A method according to claim 14, wherein said oligonucleotide is immobilized on a solid support.

19. A method for separating protected from fully deprotected synthetic oligonucleotides, comprising:

contacting a mixture of protected from fully deprotected synthetic oligonucleotides to antibodies according to claim 1, wherein said protected synthetic oligonucleotides have said organic protecting group covalently bound thereto, so that said protected synthetic oligonucleotides bind to said antibody; and then

separating said antibodies from said fully deprotected synthetic oligonucleotides.

20. A method according to claim 19, wherein said antibody is immobilized on a solid support.

21. A method according to claim 19, wherein said protected synthetic oligonucleotide is a partially protected synthetic oligonucleotide.

22. A method according to claim 19, wherein said contacting and separating steps are carried out by affinity chromatography.

23. An article useful for the determining incomplete deprotection of a synthetic oligonucleotide in an immunoassay, said article comprising:

a solid support having a surface portion, said surface portion having at least two separate discrete regions formed thereon;

a first oligonucleotide bound to one of said separate discrete regions, said first oligonucleotide having a protecting group bound thereto; and

a second oligonucleotide bound to another of said separate discrete regions, said second oligonucleotide not having said protecting group bound thereto;

wherein the nucleotide sequence of said first and second oligonucleotides are the same.

24. An article according to claim 23, further comprising:

a third oligonucleotide bound to another of said separate discrete regions; said third oligonucleotide also having said protecting group bound to said first oligonucleotide bound thereto;

wherein said third oligonucleotide is partially deprotected;

and wherein the nucleotide sequence of said first, second, and third oligonucleotides are the same.

25. An article according to claim 23, wherein said substrate comprises a nitrocellulose strip.

26. A method of making an antibody that specifically binds to a synthetic oligonucleotide having a organic protecting group covalently bound thereto, which antibody does not bind to the said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto, said method comprising the steps of:

synthesizing said synthetic oligonucleotide on a solid particulate support with said organic protecting group covalently bound to said synthetic oligonucleotide, or synthesizing a nucleotide on said solid support with said organic protecting group bound to said nucleotide; and then, without removing said oligonucleotide or nucleotide from said solid support;

immunizing an animal with said synthetic oligonucleotide or nucleotide bound to said solid support in an amount sufficient to produce said antibody.

27. A method according to claim 26, wherein said synthesizing step is followed by the step of fragmenting said beads prior to said immunizing step.

28. A method according to claim 26, further comprising the step of:

collecting said antibody from said animal.

29. A method according to claim 26, further comprising the steps of:

collecting spleen cells from said animal; then

producing a plurality of hybridoma cell lines from said spleen cells; and then

isolating a particular hybridoma cell line that produces said antibody from said plurality of hybridoma cell lines.

30. A method according to claim 26, wherein said synthetic oligonucleotide is covalently bound to said solid support.

31. A method according to claim 26, wherein said synthetic oligonucleotide is covalently bound to said solid support with a succinyl linker.

32. A method according to claim 26, wherein said solid support comprises a controlled pore glass bead.

33. A method of screening an oligonucleotide array for insufficient deprotection or insufficient elongation of oligonucleotides therein, said method comprising the steps of:

(a) providing an oligonucleotide array comprising a substrate having a plurality of different oligonucleotides immobilized thereon, with said different oligonucleotides immobilized in different separate and discrete locations on said substrate;

(b) providing an antibody that specifically binds to a synthetic oligonucleotide having an organic protecting group covalently bound thereto, which antibody does not bind to said synthetic oligonucleotide when said organic protecting group is not covalently bound thereto; and then

(c) contacting said antibody to said oligonucleotide array to thereby detect the presence or absence of binding of said antibody selected and discrete locations on

said array, the presence of binding to separate and discrete locations in said array indicating insufficient deprotection or insufficient elongation of oligonucleotides therein.

34. A method according to claim 33, wherein said substrate comprises silicon.

35. A method according to claim 33, wherein said step of providing an array is carried out by synthesizing said oligonucleotides *in situ* on said substrate.

36. A method according to claim 33, further comprising repeating steps *(b)* to *(c)* at least once with a different antibody on each repetition so that a plurality of different protecting groups on oligonucleotides in the array may be detected.

37. A method according to claim 33, further comprising the step of:

generating an indicia recording the presence of insufficient deprotection or insufficient elongation of oligonucleotides in at least one separate and discrete location on said array.

38. A method according to claim 37, wherein said indicia is a qualitative indicia.

39. A method according to claim 37, wherein said indicia is a quantitative indicia.

40. A correctable oligonucleotide array, comprising, in combination:

(a) a substrate having a plurality of different oligonucleotides immobilized thereon, with said different oligonucleotides immobilized in different separate and discrete locations on said substrate; and

(b) a plurality of indicia associated with said array, said indicia recording the presence of insufficient deprotection or insufficient elongation of at least one oligonucleotide, each of said at least one oligonucleotide located in a different separate and discrete location on said array.

41. An array according to claim 40, wherein said substrate has at least 1000 different oligonucleotides immobilized in different separate and discrete locations on said substrate.

42. An array according to claim 40, wherein said indicia are stored in or printed on said array.

43. An array according to claim 40, wherein said indicia are contained in a computer file, said array further comprising an identifier associating said substrate and said indicia.

44. An array according to claim 40, wherein said indicia are contained on a web site, said array further comprising an identifier associating said substrate and said indicia.

45. A method of using an oligonucleotide array and compensating for insufficient deprotection or insufficient elongation of oligonucleotides on said array, comprising the steps of:

(a) providing a substrate having a plurality of different oligonucleotides immobilized thereon, with said different oligonucleotides immobilized in different separate and discrete locations on said substrate;

(b) providing indicia associated with said array, said indicia recording the presence of insufficient deprotection or insufficient elongation of at least one oligonucleotide, said at least one oligonucleotide located in a separate and discrete locations on said array;

(c) providing a test compound;

(d) detecting the binding of said test compound to at least one of said plurality of different oligonucleotides; and then

(d) determining the degree of binding of said test compound to said oligonucleotide from *(i)* said detected binding and *(ii)* said indicia recording the presence of insufficient deprotection or insufficient elongation, so that said

insufficient deprotection or insufficient elongation is compensated for during said determining step.

46. A method according to claim 45, wherein said test compound is a protein, peptide, or oligonucleotide.

47. A method according to claim 45, wherein said test compound is mRNA.

48. A method according to claim 45, wherein said determining step is carried out by generating a color indication of degree of binding.

49. A method according to claim 45, wherein said determining step is carried out by generating a numeric indication of degree of binding.

50. A method according to claim 45, wherein said degree of binding is binding affinity, binding amount, or both binding affinity and binding amount.

51. A method of using an oligonucleotide array while compensating for insufficient deprotection or insufficient elongation of oligonucleotides on said array, said method comprising the steps of:

(a) providing a substrate having a plurality of different oligonucleotides immobilized thereon, with said different oligonucleotides immobilized in different separate and discrete locations on said substrate;

(b) providing indicia associated with said array, said indicia recording the presence of insufficient deprotection or insufficient elongation of at least one oligonucleotide, said at least one oligonucleotide located in a separate and discrete locations on said array;

(c) providing a test compound;

(d) contacting said test compound to said array;

(d) deleting from analysis said at least one oligonucleotide in a separate and discrete location having insufficient deprotection, with binding of said test compound

to said array being detected with the remaining oligonucleotides in separate and discrete locations that have not been deleted from analysis; and then

(d) detecting the binding of said test compound to said remaining oligonucleotides in separate and discrete locations in said array.

52. A method according to claim 51, wherein said test compound is a protein, peptide, or oligonucleotide.

53. A method according to claim 51, wherein said test compound is mRNA.

54. A method according to claim 51, wherein said detecting step is carried out by generating a color indication of binding.

55. A method according to claim 51, wherein said detecting step is carried out by generating a numeric indication of binding.

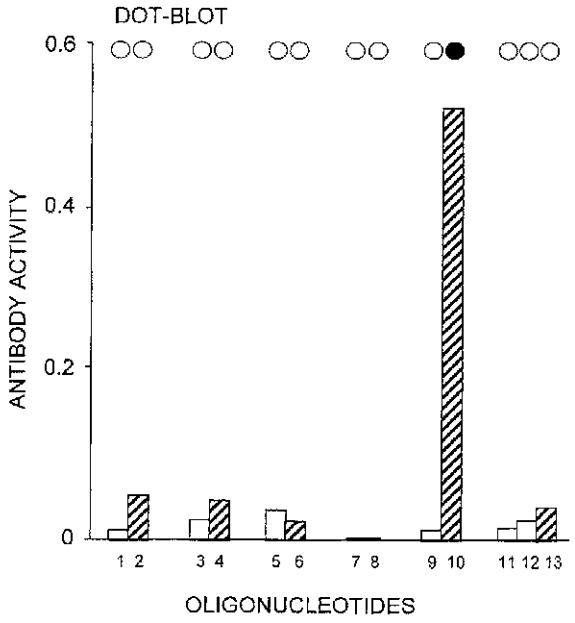

DOT-BLOT
0.6
0.4
0.2
0
ANTIBODY ACTIVITY
1 2
3 4
5 6
7 8
9 10
11 12 13
OLIGONUCLEOTIDES

FIG. 1

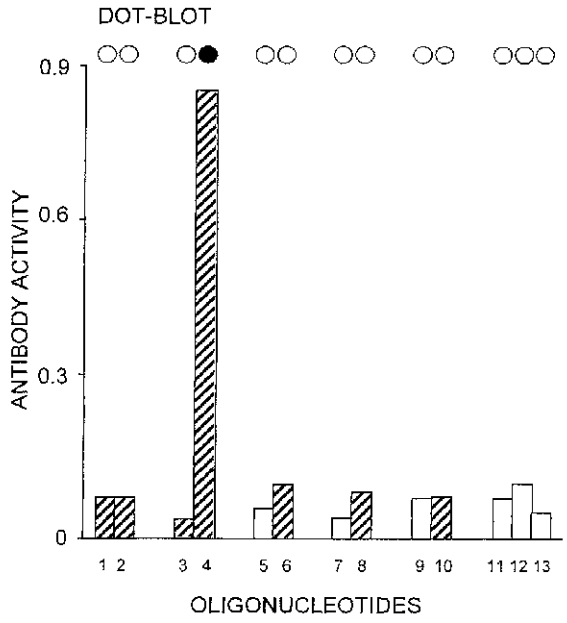

DOT-BLOT
0.9
0.6
0.3
0
ANTIBODY ACTIVITY
1 2
3 4
5 6
7 8
9 10
11 12 13
OLIGONUCLEOTIDES

FIG. 2

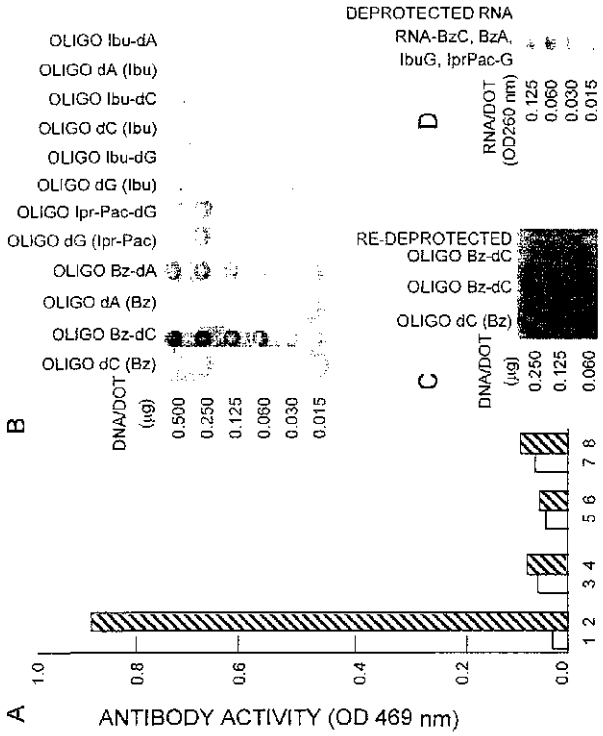

DEPROTECTED RNA
RNA-BzC, BzA,
IbuG, IprPac-G
D
RNA/DOT
(OD260 nm)
0.125
0.060
0.030
0.015
RE-DEPROTECTED
OLIGO Bz-dC
OLIGO Bz-dC
OLIGO dC (Bz)
C
DNA/DOT
(µg)
0.250
0.125
0.060
OLIGO Ibu-dA
OLIGO dA (Ibu)
OLIGO Ibu-dC
OLIGO dC (Ibu)
OLIGO Ibu-dG
OLIGO dG (Ibu)
OLIGO Ipr-Pac-dG
OLIGO dG (Ipr-Pac)
OLIGO Bz-dA
OLIGO dA (Bz)
OLIGO Bz-dC
OLIGO dC (Bz)
B
DNA/DOT
(µg)
0.500
0.250
0.125
0.060
0.030
0.015
1 2 3 4 5 6 7 8
1.0 0.8 0.6 0.4 0.2 0.0
A
ANTIBODY ACTIVITY (OD 469 nm)

FIG. 3
3/10

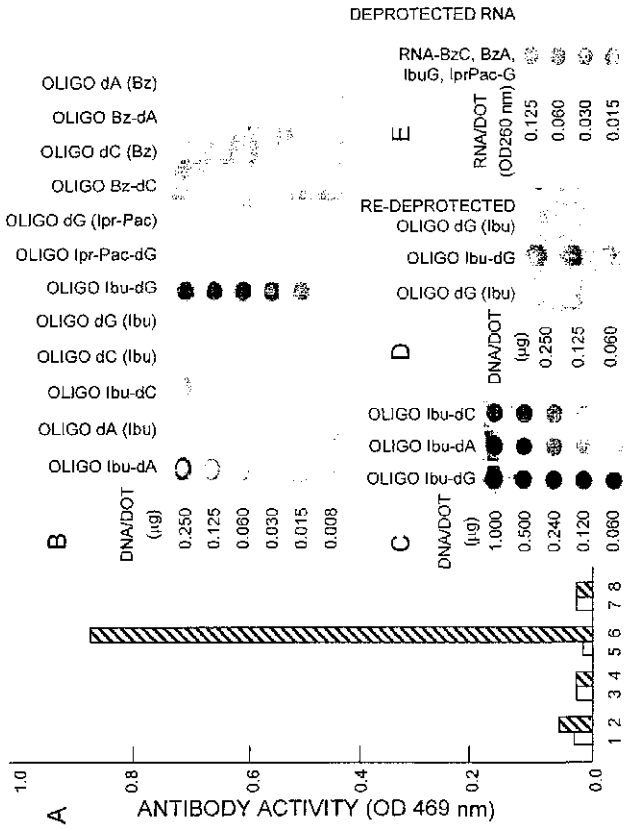

DEPROTECTED RNA
OLIGO dA (Bz)
OLIGO Bz-dA
OLIGO dC (Bz)
OLIGO Bz-dC
OLIGO dG (Ipr-Pac)
OLIGO Ipr-Pac-dG
OLIGO Ibu-dG
OLIGO dG (Ibu)
OLIGO dC (Ibu)
OLIGO Ibu-dC
OLIGO dA (Ibu)
OLIGO Ibu-dA
B
DNA/DOT (µg)
0.250
0.125
0.060
0.030
0.015
0.008
C
DNA/DOT (µg)
1.000
0.500
0.240
0.120
0.060
OLIGO Ibu-dC
OLIGO Ibu-dA
OLIGO Ibu-dG
RE-DEPROTECTED
OLIGO dG (Ibu)
OLIGO Ibu-dG
OLIGO dG (Ibu)
D
DNA/DOT (µg)
0.250
0.125
0.060
RNA-BzC, BzA, IbuG, IprPac-G
E
RNA/DOT (OD260 nm)
0.125
0.060
0.030
0.015
1 2 3 4 5 6 7 8
1.0 0.8 0.6 0.4 0.2 0.0
A
ANTIBODY ACTIVITY (OD 469 nm)

FIG. 4
4/10

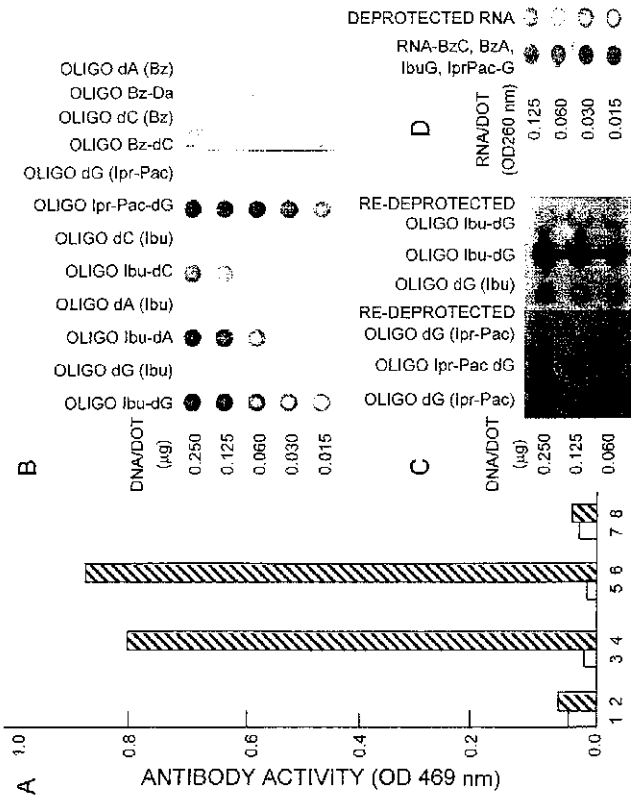

DEPROTECTED RNA
RNA-BzC, BzA, IbuG, IprPac-G
D
RNA/DOT (OD260 nm)
0.125
0.060
0.030
0.015
OLIGO dA (Bz)
OLIGO Bz-Da
OLIGO dC (Bz)
OLIGO Bz-dC
OLIGO dG (Ipr-Pac)
OLIGO Ipr-Pac-dG
OLIGO dC (Ibu)
OLIGO Ibu-dC
OLIGO dA (Ibu)
OLIGO Ibu-dA
OLIGO dG (Ibu)
OLIGO Ibu-dG
RE-DEPROTECTED OLIGO Ibu-dG
OLIGO Ibu-dG
OLIGO dG (Ibu)
RE-DEPROTECTED OLIGO dG (Ipr-Pac)
OLIGO Ipr-Pac dG
OLIGO dG (Ipr-Pac)
B
DNA/DOT (µg)
0.250
0.125
0.060
0.030
0.015
C
DNA/DOT (µg)
0.250
0.125
0.060
1 2
3 4
5 6
7 8
A
1.0
0.8
0.6
0.4
0.2
0.0
ANTIBODY ACTIVITY (OD 469 nm)

FIG. 5

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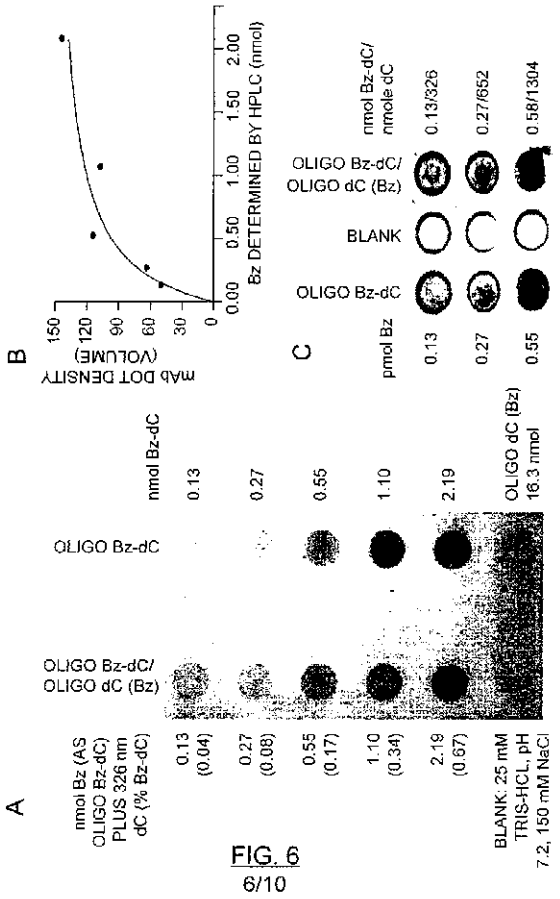
A
nmol Bz (AS OLIGO Bz-dC) PLUS 326 nm dC (% Bz-dC)
0.13 (0.04)
0.27 (0.08)
0.55 (0.17)
1.10 (0.34)
2.19 (0.67)
BLANK: 25 mM TRIS-HCL, pH 7.2, 150 mM NaCl
OLIGO Bz-dC/ OLIGO dC (Bz)
OLIGO Bz-dC
nmol Bz-dC
0.13
0.27
0.55
1.10
2.19
OLIGO dC (Bz) 16.3 nmol
B
mAb DOT DENSITY (VOLUME)
150
120
90
60
30
0
0.00
0.50
1.00
1.50
2.00
Bz DETERMINED BY HPLC (nmol)
C
pmol Bz
0.13
0.27
0.55
OLIGO Bz-dC
BLANK
OLIGO Bz-dC/ OLIGO dC (Bz)
nmol Bz-dC/ nmole dC
0.13/326
0.27/652
0.58/1304

FIG. 6

6/10

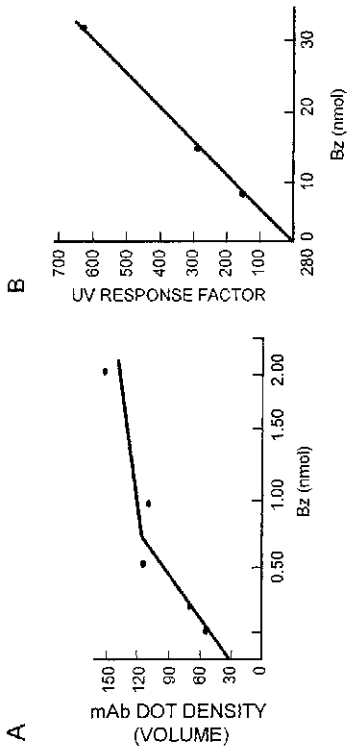
B
700
600
500
400
300
200
100
280
UV RESPONSE FACTOR
0
10
20
30
Bz (nmol)
A
150
120
90
60
30
0
mAb DOT DENSITY
(VOLUME)
0.50
1.00
1.50
2.00
Bz (nmol)

FIG. 7

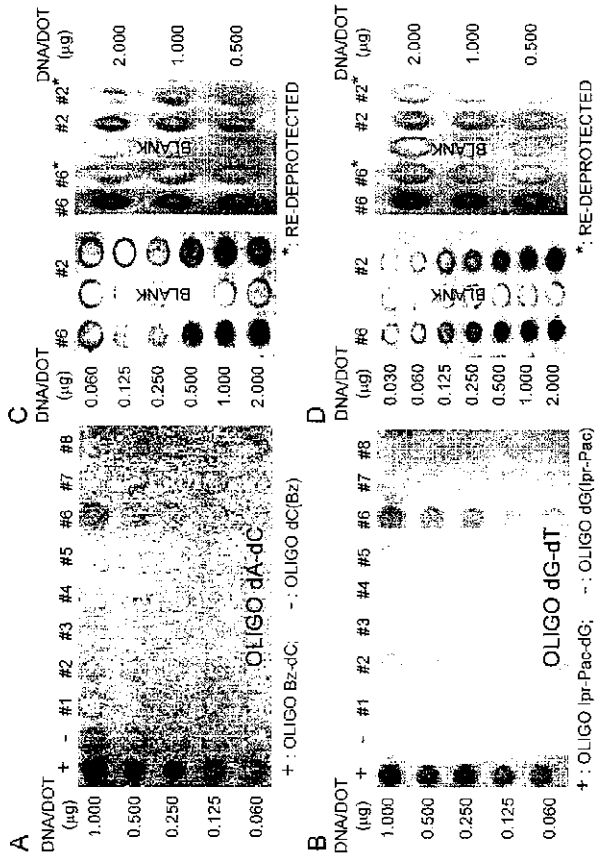
A
B
C
D
OLIGO dA-dC
OLIGO dG-dT
BLANK
+ : OLIGO Bz-dC; - : OLIGO dC(Bz)
+ : OLIGO Ipr-Pac-dG; - : OLIGO dG(Ipr-Pac)
*: RE-DEPROTECTED

FIG. 8

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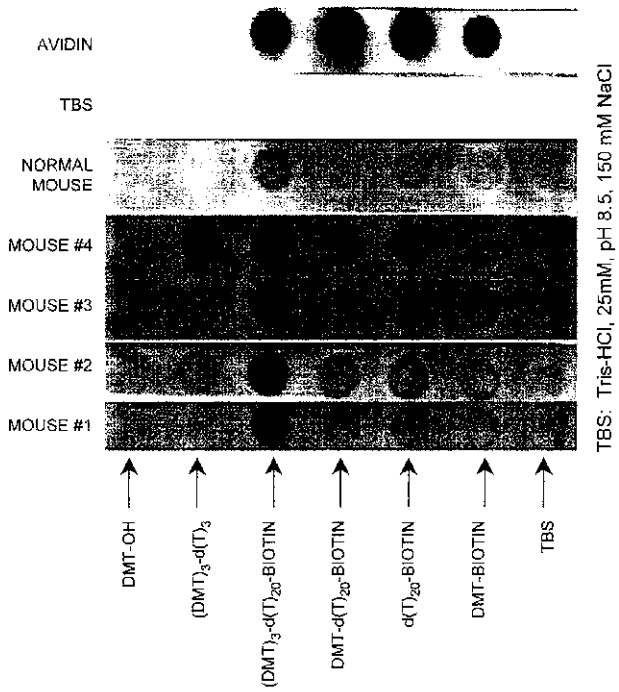
AVIDIN
TBS
NORMAL MOUSE
MOUSE #4
MOUSE #3
MOUSE #2
MOUSE #1
TBS: Tris-HCl, 25mM, pH 8.5, 150 mM NaCl
DMT-OH
(DMT)3-d(T)3
(DMT)3-d(T)20-BIOTIN
DMT-d(T)20-BIOTIN
d(T)20-BIOTIN
DMT-BIOTIN
TBS

FIG. 9

9/10

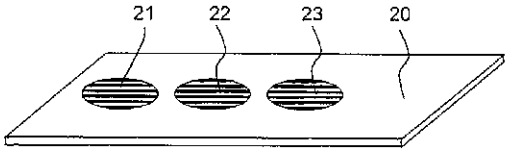

FIG. 10

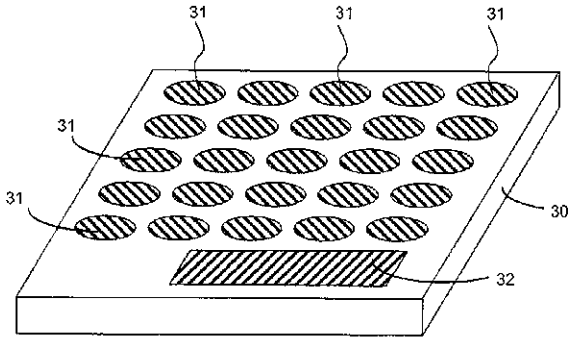

FIG. 11

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/35600

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :Please See Extra Sheet.
US CL :436/6; 436/527, 547; 530/388.21, 413
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)
U.S. : Please See Extra Sheet.

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 practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,049,656 A (LEWIS et al) 17 September 1991, see entire document.	1-3, 7-15, 18-24, 40-42, 45-47, 50 ---------- 4-6, 16,17, 25-39, 43, 44, 48, 49, 51-55

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"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
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 MARCH 2001	17 APR 2001

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	JAMES L. GRUN, PH.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/35600

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,221,736 A (COOLIDGE et al) 22 JUNE 1993, see entire document.	1-3, 7-15, 18-24, 40-42, 45-47, 50 --- 4-6, 16,17, 25-39, 43, 44, 48, 49, 51-55
Y	US 5,871,747 A (GENGOUX-SEDLIK et al) 16 February 1999, see entire document.	26-32
Y	DEGLING et al. Biodegradable Microshperes XVIII: The Adjuvant Effect of Polyacryl Starch Microparticles with Conjugated Human Serum Albumin. Vaccine. 1995, Volume 13, No. 7, pages 629-636, see entire document	26-32

Form PCT/ISA/210 (continuation of second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/35600

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07K 16/18; C12P 21/08; C12Q 1/68; G01N 33/53, 33/531, 33/532, 33/577

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

422/50, 56, 57, 61; 424/184.1, 278.1; 435/6, 7.1, 7.92, 7.94; 436/518, 524, 527, 528, 547, 548, 72, 164, 169, 807, 809; 530/388.21, 389.1, 391.1, 413

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

EAST; DIALOG
search terms: antibod?, (pac or ipr or dnrf or se or dmt or bx or ibu), (immunize or immunization), (bead or microparticle or particle), (nucleic or oligonucleotide), (protect? or block? or cap? or modif?)

Form PCT/ISA/210 (extra sheet) (July 1998)*

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(51)Int.Cl.[7]	FI		テーマコード（参考）
Ｃ１２Ｑ 1/68	Ｇ０１Ｎ 30/88	Ｅ	４Ｈ０４５
Ｇ０１Ｎ 30/48	Ｇ０１Ｎ 33/53	Ｍ	
Ｇ０１Ｎ 30/88	Ｇ０１Ｎ 37/00	１０２	
Ｇ０１Ｎ 33/53	Ｃ１２Ｎ 5/00	Ｂ	
Ｇ０１Ｎ 37/00	Ｃ１２Ｎ 15/00	Ｆ	

(81)指定国 AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CR,CU,CZ,DE,DK,DM,DZ,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,US,UZ,VN,YU,ZA,ZW

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Ｆターム(参考) 4B024 AA11 BA41 CA01 CA12 HA12 HA15
4B029 AA07 AA23 BB15 BB20 FA01 FA12 FA15
4B063 QA01 QA18 QQ42 QQ53 QQ79 QQ96 QR32 QR48 QR55 QR82
QS33 QS34 QX01
4B064 AG27 CA19 CC24 DA13
4B065 AA90X AA92X AC14 BA08 CA25 CA46
4H045 AA11 BA10 CA40 DA76 EA50 FA74

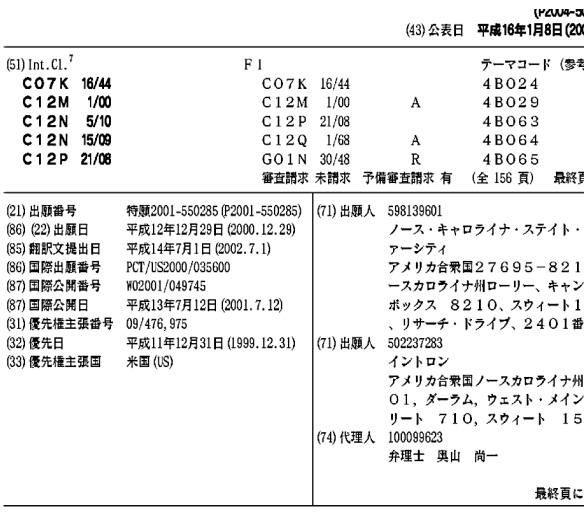
专利名称(译)	用于测量化学合成的核酸的纯度的方法和组合物

公开(公告)号	JP2004500370A	公开(公告)日	2004-01-08
申请号	JP2001550285	申请日	2000-12-29

[标]申请(专利权)人(译)	北卡罗莱纳州立大学 内含子
申请(专利权)人(译)	北卡罗来纳州盐湖城 内含子
[标]发明人	アグリスポールエフ ミッチェルロイドジー ピアースクリストファーディージェイ
发明人	アグリス,ポール·エフ ミッチェル,ロイド·ジー ピアース,クリストファー·ディー·ジェイ
IPC分类号	G01N33/53 B01J20/281 C07K16/18 C07K16/44 C12M1/00 C12N5/10 C12N15/09 C12P21/08 C12Q1/68 G01N30/88 G01N37/00 G01N30/48
CPC分类号	C07K16/44 B01J2219/00274 C07K16/18 C12Q1/6804 Y02P20/55
FI分类号	C07K16/44 C12M1/00.A C12P21/08 C12Q1/68.A G01N30/48.R G01N30/88.E G01N33/53.M G01N37/00.102 C12N5/00.B C12N15/00.F
F-TERM分类号	4B024/AA11 4B024/BA41 4B024/CA01 4B024/CA12 4B024/HA12 4B024/HA15 4B029/AA07 4B029/AA23 4B029/BB15 4B029/BB20 4B029/FA01 4B029/FA12 4B029/FA15 4B063/QA01 4B063/QA18 4B063/QQ42 4B063/QQ53 4B063/QQ79 4B063/QQ96 4B063/QR32 4B063/QR48 4B063/QR55 4B063/QR82 4B063/QS33 4B063/QS34 4B063/QX01 4B064/AG27 4B064/CA19 4B064/CC24 4B064/DA13 4B065/AA90X 4B065/AA92X 4B065/AC14 4B065/BA08 4B065/CA25 4B065/CA46 4H045/AA11 4H045/BA10 4H045/CA40 4H045/DA76 4H045/EA50 4H045/FA74
优先权	09/476975 1999-12-31 US
外部链接	Espacenet

摘要(译)

本申请具体描述了特异性结合有机保护基共价连接的合成低聚物（例如，寡核苷酸或寡肽）的抗体，并且如果有机保护基不共价连接则不与合成低聚物结合。它被描述。还公开了制备和使用此类抗体的方法，包含此类抗体的细胞，以及具有固定化寡聚体的制品，其可用于使用此类抗体的测定程序。



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