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Families of non-cross-hybridizing polynucleotides for use as tags and tag complements, manufacture and use thereof
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## COMPLETE SPECIFICATION

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ORIGINAL
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Title:
POLYNUCLEOTIDES FOR USE AS TAGS AND TAG COMPLEMENTS, MANUFACTURE AND USE THEREOF
Details of Original Application No. 2002227829 dated 25 Jan 2002
The following statement is a full description of this invention, including the best method of performing it known to me/us:-

File: 39709AUP01

## FAMILIES OF NON-CROSS - HYBRIDIZING POLYNUCLEOTIDES FOR USE AS TAGS AND TAG COMPLEMENTS, MANUFACTURE AND USE THEREOF

 2002227829 , which is incorporated in its entirety herein by reference.
## FIELD OF THE INVENTION

This invention relates to families of oligonucleotide tags for use, for example, in sorting molecules. Members of a given family of tags can be distinguished one from the other by specific hybridization to their tag complements.

## BACKGROUND OF THE INVENTION

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Specific hybridization of oligonucleotides and their analogs is a fundamental process that is employed in a wide variety of research, medical, and industrial applications, including the identification of disease-related polynucleotides in diagnostic assays, screening for clones of novel target polynucleotides, identification of specific polynucleotides in blots of mixtures of polynucleotides, therapeutic blocking of inappropriately expressed genes and DNA sequencing. Sequence specific hybridization is critical in the development of high throughput multiplexed nucleic acid assays. As formats for these assays expand to encompass larger amounts of sequence information acquired through projects such as the Human Genome project, the challenge of sequence specific hybridization with high fidelity is becoming increasingly difficult to achieve.

In large part, the success of hybridization using oligonucleotides depends on minimizing the number of false positives and false negatives. Such problems have made the simultaneous use of multiple hybridization probes in a single experiment i. e. multiplexing, particularly in the analysis of multiple gene sequences on a gene microarray, very difficult. For example, in certain binding assays, a number of nucleic acid molecules are bound to a chip with the desire that a given "target" sequence will bind selectively to its complement attached to the chip. Approaches have been developed that involve the use of oligonucleotide tags attached to a solid support that
can be used to specifically hybridize to the tag complements that are coupled to probe sequences. Chetverin et al. (WO 93/17126) uses sectioned, binary oligonucleotide arrays to sort and survey nucleic acids. These arrays have a constant nucleotide sequence attached to an adjacent variable nucleotide sequence, both bound to a solid 5 support by a covalent linking moiety. These binary arrays have advantages compared with ordinary arrays in that they can be used to sort strands according to their terminal sequences so that each strand binds to a fixed location on an array. The design of the terminal sequences in this approach comprises the use of constant and variable $\qquad$

sequences. United States Patent Nos. $6,103,463$ and $6,322,971$ issued to Chetverin et al. on August 15, 2000 and November 27, 2001, respectively. This concept of using molecular tags to sort a mixture of molecules is analogous to molecular tags developed for bacterial and yeast genetics (Hensel et al., Science; 269, 400-403: 1995 and Schoemaker et al., Nature Genetics; 14, 450-456: 1996). Here, a method termed "signature tagged" mutagenesis in which each mutant is tagged with a different DNA sequence is used to recover mutant genes from a complex mixture of approximately 10,000 bacterial colonies. In the tagging approach of Barany et al. (WO 9731256), known as the "zip chip", a family of nucleic acid molecules, the "zip-code addresses", each different from each other, are set out on a grid. Target molecules are attached to oligonucleotide sequences complementary to the "zipcode addresses," referred to as "zipcodes," which are used to specifically hybridize to the address locations on the grid. While the selection of these families of polynucleotide sequences used as addresses is critical for correct performance of the assay, the performance has not been described.

Working in a highly parallel hybridization environment requiring specific hybridization imposes very rigorous selection criteria for the design of families of oligonucleotides that are to be used. The success of these approaches is dependent on the specific hybridization of a probe and its complement. Problems arise as the family of nucleic acid molecules crosshybridize or hybridize incorrectly to the target sequences. While it is common to obtain incorrect hybridization resulting in false positives or an inability to form hybrids resulting in false negatives, the frequency of such results must be minimized. In order to achieve this goal certain thermodynamic properties of forming nucleic acid hybrids must be considered. The temperature at which oligonucleotides form duplexes with their complementary sequences known as the $T_{m}$ (the temperature at which $50 \%$ of the nucleic acid duplex is dissociated) varies according to a number of sequence dependent properties including the hydrogen bonding energies of the canonical pairs A-T and G-C (reflected in GC or base composition), stacking free energy and, to a lesser extent, nearest neighbour interactions. These energies vary widely among oligonucleotides that are typically used in hybridization assays. For example, hybridization of two probe sequences composed of 24 nucleotides, one with a $40 \%$ GC content and the other with a $60 \%$ GC content, with its complementary target under standard conditions theoretically may have a $10^{\circ} \mathrm{C}$ difference in melting temperature (Mueller et al., Current Protocols in Mol. Biol.; 15, 5:1993). Problems in hybridization occur when the hybrids are allowed to form under hybridization conditions that include a single hybridization temperature that is not optimal for correct hybridization of all
oligonucleotide sequences of a set. Mismatch hybridization of noncomplementary probes can occur forming duplexes with measurable mismatch stability (Santalucia et al., Biochemistry; 38: 3468-77, 1999). Mismatching of duplexes in a particular set of oligonucleotides can occur under hybridization conditions where the mismatch results in a decrease in duplex stability that results in a higher $T_{m}$ than the least stable correct duplex of that particular set. For example, if hybridization is carried out under conditions that favor the AT-rich perfect match duplex sequence, the possibility exists for hybridizing a GC-rich duplex sequence that contains a mismatched base having a melting temperature that is still above the correctly formed AT-rich duplex. Therefore, design of families of oligonucleotide sequences that can be used in multiplexed hybridization reactions must include consideration for the thermodynamic properties of oligonucleotides and duplex formation that will reduce or eliminate cross hybridization behavior within the designed oligonucleotide set.

The development of such families of tags has been attempted over the years with varying degrees of success. There are a number of different approaches for selecting sequences for use in multiplexed hybridization assays. The selection of sequences that can be used as zipcodes or tags in an addressable array has been described in the patent literature in an approach taken by Brenner and co-workers. United States Patent No. 5, 654,413 describes a population of oligonucleotide tags (and corresponding tag complements) in which each oligonucleotide tag includes a plurality of subunits, each subunit consisting of an oligonucleotide having a length of from three to six nucleotides and each subunit being selected from a minimally cross hybridizing set, wherein a subunit of the set would have at least two mismatches with any other sequence of the set. Table II of the Brenner patent specification describes exemplary groups of 4 mer subunits that are minimally cross hybridizing according to the aforementioned criteria. In the approach taken by Brenner, constructing non cross-hybridizing oligonucleotides, relies on the use of subunits that form a duplex having at least two mismatches with the complement of any other subunit of the same set. The ordering of subunits in the construction of oligonucleotide tags is not specifically defined.
parameters used in the design of tags based on subunits are discussed in Barany et al. (wo 9731256). For example, in the design of polynucleotide sequences that are for example 24 nucleotides in length ( 24 mer) derived from a set of four possible tetramers in which each 24 mer "address" differs from its nearest 24 mer neighbour by 3 tetramers. They discuss further that, if each tetramer differs from each other by at least two nucleotides, then each 24 mer will differ from the next by at least six nucleotides. This is determined
without consideration for insertions or deletions when forming the alignment between any two sequences of the set. In this way a unique "zip code" sequence is generated. The zip code is ligated to a label in a target dependent manner, resulting in a unique "zip code" which is then allowed to "zip code" to other "addresses", the hybridization reaction is carried out at temperatures of $75-80^{\circ} \mathrm{C}$. Due to the high temperature conditions for hybridization, 24 mers that have partial homology hybridize to a lesser extent than sequences with perfect complementarity and represent 'dead zones'. This approach of implementing stringent hybridization conditions for example, involving high temperature hybridization, is also practiced by Brenner et. al.

The current state of technology for designing non-cross hybridizing tags based on subunits does not provide sufficient guidance to construct a family of relatively large numbers of sequences with practical value in assays that require stringent non-cross hybridizing behavior.

A multiplex sequencing method has been described in United States Patent No. 4,942,124, which issued to Church on July 17, 1990. The method requires at least two vectors which differ from each other at a tag sequence. It is stated that a tag sequence in one vector will not hybridize under stringent hybridization conditions to a tag sequence (i.e., complementary probes do not cross-hybridize) in another vector. Exemplary stringent hybridization conditions are given as $42^{\circ} \mathrm{C}$ in $500-$ 1000 mM sodium phosphate buffer. A set of 42 20-mer tag sequences, all of which lack $G$ residues, is given in Figure 3 of the specification. Details of how the sequences were obtained are not provided, although Church states that initially 92 were chosen on the basis of their having sufficient sequence diversity to insure uniqueness.

So while it is possible for a person knowledgeable in the field to design a small number of non-cross hybridizing tags, it is difficult to design a larger number such tags. A co-pending application of the owner of this patent application describes such a set of 210 non-cross hybridizing tags that have a practical value. A method described in international patent application No. PCT/CA 01/00141 published under wo $01 / 59151$ on August 16, 2001. Little guidance is provided, however, for the provision of a larger set, say 1000 or so, of non-cross hybridizing tags. Since having sets of approximately 1000 non-cross hybridizing tags, or more, would be of considerable practical value, it would be useful to develop such a set.

Thus, while it is desirable with such arrays to have, at once, a large number of address molecules, the address molecules should each be highly selective for its own complement sequence. While such an array provides the
advantage that the family of molecules making up the grid is entirely of design, and does not rely on sequences as they occur in nature, the provision of a family of molecules, which is sufficiently large and where each individual member is sufficiently selective for its complement over all the other zipcode molecules (i.e., where there is sufficiently low cross-hybridization, or cross-talk) continues to elude researchers.

## SUMMARY OF INVENTION

According to a first aspect of the invention there is provided a composition comprising at least one hundred and fifty minimally cross-hybridizing molecules for use as tags or tag complements wherein each molecule comprises an oligonucleotide comprising a sequence of nucleotide bases for which, under a defined set of conditions, the maximum degree of hybridization between said oligonucleotide and any complement of a different oligonucleotide does not exceed about $20 \%$ of the degree of hybridization between said oligonucleotide and a complement to said oligonucleotide, wherein for each said sequence there is at most six bases other than $G$ between every pair of neighbouring pairs of G's, and wherein the oligonucleotides are selected from SEQ ID NOs 1-1168, or the complete complements of SEQ ID NOs 1-1168.

According to a second aspect of the invention there is provided a composition comprising a plurality of minimally cross-hybridizing oligonucleotide tag complements, each oligonucleotide of each tag complement comprising:
(a) each oligonucleotide is free of either cytosine or guanosine residues;
(b) no two cytosine or guanosine residues are located adjacent each other in an oligonucleotide and any two cytosine or guanosine residues are separated by at most 6 non-cytosine or non-guanosine residues, respectively;
(c) the number of cytosine or guanosine residues in each oligonucleotide does not exceed $\mathrm{L} / 4$ where L is the number of bases in the oligonucleotide;
(d) the length of each oligonucleotide differs by no more than five bases from the average length of all oligonucleotides in the composition;
(e) each oligonucleotide does not contain 4 or more contiguous identical nucleotides;
(f) the number of guanosine or cytosine residues in each oligonucleotide does not vary from the average number of guanosine or cytosine residues in all other oligonucleotides of the composition by more than one; and
(g) when each oligonucleotide tag complement is exposed to hybridization conditions comprising $0.2 \mathrm{M} \mathrm{NaCl}, 0.1 \mathrm{M}$ Tris, $0.08 \%$ Triton $\mathrm{X}-100, \mathrm{pH} 8.0$ at $37^{\circ} \mathrm{C}$, the maximum degree of hybridization between the tag complement and a tag not fully complementary to the tag complement does not exceed $30 \%$ of the degree of hybridization between the tag complement and its fully complementary tag; wherein the oligonucleotides are selected from SEQ ID NOs 1-1168, or the complete complements of SEQ ID NOs 1-1168.

According to a third aspect of the invention there is provided a kit for sorting and identifying polynucleotides, the kit comprising one or more solid phase supports each having one or more spatially discrete regions, each such region having a uniform population of substantially identical tag complements covalently attached, and the tag complements each being selected from the set of oligonucleotides as defined in the first aspect.

Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

A family of 1168 sequences was obtained using a computer algorithm to have desirable hybridization properties for use in nucleic acid detection assays. The sequence set of 1168 oligonucleotides was partially characterized in hybridization assays, demonstrating the ability of family members to correctly hybridize to their complementary sequences with minimal cross hybridization. These are the sequences having SEQ ID NOs: 1 to 1168 of Table I.

Variant families of sequences (seen as tags or tag complements) of a family of sequences taken from Table I are also part of the invention. For the purposes of discussion, a family or set of oligonucleotides will often be described as a family of tag complements, but it will be understood that such a set could just easily be a family of tags.

A family of complements is obtained from a set of oligonucleotides based on a family of oligonucleotides such as those of Table I. To simplify discussion, providing a family of complements based on the oligonucleotides of Table I will be described.

Firstly, the groups of sequences based on the oligonucleotides of Table I can be represented as shown in Table IA.

Table IA: Numeric sequences corresponding to nucleotide base patterns of a set of oligonucleotides

Sequence
Identifier Identifier

| 1 | 1 | 1 | 2 | 2 | 3 | 2 | 3 | 1 | 1 | 1 | 3 | 1 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 1 |  | 1 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |

$\begin{array}{llllllllllllllllllllllllll}3 & 2 & 2 & 1 & 3 & 1 & 3 & 2 & 2 & 1 & 1 & 2 & 2 & 3 & 2 & 1 & 2 & 2 & 2 & 3 & 1 & 2 & 3 & 1 & & 2\end{array}$
$\begin{array}{lllllllllllllllllllllllllll}1 & 2 & 3 & 2 & 2 & 1 & 1 & 1 & 3 & 2 & 1 & 1 & 3 & 2 & 3 & 2 & 2 & 3 & 1 & 1 & 1 & 2 & 3 & 2 & 3\end{array}$
$\begin{array}{lllllllllllllllllllllllllll}10 & 2 & 3 & 1 & 2 & 3 & 2 & 2 & 1 & 3 & 1 & 1 & 3 & 2 & 1 & 2 & 1 & 2 & 2 & 3 & 2 & 3 & 1 & 1 & 2 & 4\end{array}$
$\begin{array}{lllllllllllllllllllllllll}2 & 2 & 2 & 3 & 2 & 3 & 2 & 1 & 3 & 1 & 1 & 2 & 1 & 2 & 3 & 2 & 3 & 2 & 2 & 3 & 2 & 2 & 1 & 1 & 5\end{array}$
$\begin{array}{llllllllllllllllllllllllll}1 & 2 & 1 & 1 & 3 & 2 & 3 & 2 & 1 & 1 & 3 & 2 & 3 & 1 & 1 & 1 & 2 & 1 & 1 & 3 & 1 & 1 & 3 & 1 & 6\end{array}$
$\begin{array}{lllllllllllllllllllllllllll}1 & 1 & 3 & 1 & 3 & 2 & 1 & 2 & 2 & 2 & 3 & 2 & 2 & 3 & 2 & 3 & 1 & 3 & 2 & 2 & 1 & 1 & 1 & 2 & 7\end{array}$

| 3 | 2 | 3 | 2 | 2 | 2 | 1 | 2 | 3 | 2 | 2 | 1 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 1 | 3 | 2 | 2 | 2 |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |

$\begin{array}{lllllllllllllllllllllllllll}15 & 1 & 1 & 1 & 3 & 1 & 3 & 1 & 1 & 2 & 1 & 3 & 1 & 1 & 2 & 1 & 2 & 3 & 2 & 3 & 2 & 1 & 1 & 3 & 2 & & 9\end{array}$
$\begin{array}{llllllllllllllllllllllllll}2 & 1 & 2 & 3 & 1 & 1 & 1 & 3 & 1 & 3 & 2 & 3 & 1 & 3 & 1 & 2 & 1 & 1 & 2 & 3 & 2 & 2 & 2 & 1 & 10\end{array}$
$\begin{array}{lllllllllllllllllllllllll}1 & 2 & 3 & 1 & 3 & 1 & 1 & 1 & 2 & 1 & 2 & 3 & 2 & 2 & 1 & 3 & 1 & 1 & 2 & 3 & 2 & 3 & 1 & 2 & 11\end{array}$
$\begin{array}{lllllllllllllllllllllll}2 & 2 & 1 & 3 & 2 & 3 & 2 & 2 & 3 & 1 & 2 & 3 & 2 & 2 & 2 & 1 & 3 & 2 & 1 & 3 & 2 & 2 & 2\end{array}$
$\begin{array}{llllllllllllllllllllllllll}3 & 2 & 1 & 1 & 1 & 3 & 1 & 3 & 2 & 1 & 2 & 1 & 1 & 3 & 2 & 2 & 2 & 3 & 1 & 2 & 3 & 1 & 2 & 1 & 13\end{array}$
$\begin{array}{llllllllllllllllllllllllllll}20 & 1 & 1 & 1 & 3 & 2 & 1 & 1 & 3 & 1 & 1 & 2 & 3 & 1 & 2 & 3 & 2 & 1 & 1 & 2 & 1 & 1 & 3 & 2 & 3 & & \text { I } 4\end{array}$
$\begin{array}{lllllllllllllllllllllllllll}3 & 2 & 1 & 3 & 1 & 1 & 1 & 2 & 1 & 3 & 2 & 2 & 2 & 1 & 2 & 2 & 3 & 1 & 2 & 3 & 1 & 2 & 2 & 3 & 15\end{array}$
$\begin{array}{llllllllllllllllllllllll}2 & 3 & 2 & 1 & 1 & 2 & 3 & 1 & 1 & 2 & 1 & 3 & 2 & 3 & 1 & 3 & 2 & 2 & 1 & 2 & 2 & 2 & 16\end{array}$

Table IA: Numeric sequences corresponding to nucleotide
base patterns of a set of oligonucleotides
Numeric Pattern


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| 1 | 1 | 1 |  |  |  | 3 |  | 1 | 3 | 2 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 2 | 3 | 1 | 2 | 1 | 185 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 2 | 2 | 1 | 32 | 23 | 1 | 3 | 1 | 1 | 1 | 2 | 3 | 2 | 2 | 2 | 1 | 1 | 2 | 3 | 2 | 3 | 1 | 2 | 186 |
| 2 | 3 | 1 | 1 | 31 | 1 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 1 | 1 | 2 | 2 | 1 | 3 | 2 | 2 | 2 | 3 | 187 |
| 3 | 2 | 2 | 2 | 31 | 2 | 1 | 3 | 2 | 2 | 2 | 1 | 1 | 2 | 3 | 1 | 3 | 2 | 1 | 2 | 2 | 3 | 1 | 188 |
| 3 | 2 | 2 | 3 | 21 | 1 | 3 | 2 | 1 | 1 | 2 | 3 | 1 | 2 | 1 | 1 | 1 | 3 | 2 | 1 | 2 | 3 | 1 | 189 |
| 2 | 1 | 1 | 3 | 13 | 2 | 1 | 3 | 2 | 1 | 1 | 2 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 1 | 3 | 1 | 190 |
| 2 | 2 | 2 | 3 | 13 | 1 | 3 | 1 | 3 | 2 | 1 | 2 | 3 | 2 | 1 | 2 | 3 | 1 | 2 | 2 | 1 | 2 | 2 | 191 |
| 1 | 2 | 2 | 3 | 12 | 22 | 3 | 2 | 3 | 1 | 1 | 2 | 2 | 1 | 3 | 1 | 2 | 1 | 3 | 1 | 1 | 3 | 1 | 192 |
| 3 | 1 | 2 | 2 | 13 | 32 | 1 | 2 | 2 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 1 | 2 | 1 | 3 | 1 | 3 | 193 |
| 2 | 1 | 2 | 3 | 21 | 12 | 2 | 1 | 3 | 1 | 3 | 1 | 2 | 1 | 2 | 2 | 3 | 1 | 1 | 1 | 3 | 2 | 3 | 194 |
| 2 | 1 | 2 | 3 | 23 | 31 | 1 | 1 | 3 | 2 | 1 | 1 | 2 | 3 | 1 | 2 | 1 | 1 | 1 | 2 | 3 | 1 | 3 | 195 |
| 3 | 2 | 1 | 1 | 22 | 21 | 3 | 2 | 1 | 1 | 2 | 3 | 1 | 2 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 1 | 3 | 196 |
| 3 | 2 | 2 | 2 | 12 | 22 | 3 | 2 | 1 | 1 | 1 | 3 | 1 | 2 | 3 | 2 | 1 | 1 | 3 | 2 | 3 | 1 | 1 | 197 |
| 2 | 1 | 3 | 2 | 13 | 31 | 1 | 2 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 1 | 1 | 1 | 3 | 1 | 1 | 2 | 3 | 198 |
| 2 | 1 | 2 | 2 | 32 | 22 |  | 3 | 2 | 2 | 1 | 2 | 3 | 2 | 1 | 3 | 2 | 3 | 2 | 3 | 2 | 1 | 1 | 199 |
| 3 | 1 | 3 | 2 | 3 | 11 | 1 | 3 | 2 | 2 | 1 | 2 | 1 | 2 | 3 | 1 | 1 | 1 | 3 | 2 | 1 | 2 | 1 | 0 |
| 1 | 2 | 1 | 2 | 13 | 31 | 1 | 3 | 2 | 2 | 3 | 1 | 2 | 3 | 1 | 3 | 2 | 2 | 2 | 1 | 2 | 3 | 1 | 201 |
| 2 | 2 | 2 | 1 | 3 | 11 | 1 | 2 | 1 | 1 | 3 | 1 | 1 | 2 | 1 | 1 | 3 | 2 | 3 | 1 | 3 | 2 | 1 | 202 |
| 2 | 3 | 2 | 3 | 2 | 12 | 21 | 1 | 3 | 1 | 2 | 1 | 2 | 2 | 2 | 3 | 2 | 1 | 1 | 3 | 1 | 1 | 3 | 203 |
| 2 | 1 | 3 | 1 | 1 | 31 | 13 | 2 | 2 | 3 | 2 | 1 | 2 | 2 | 3 | 2 | 2 | 1 | 2 | 1 | 1 | 3 | 2 | 204 |
| 3 | 2 | 3 | 2 | 2 | 12 | 2 | 1 | 3 | 2 | 2 | 2 | 1 | 1 | 3 | 2 | 2 | 1 | 3 | 1 | 3 | 2 | 1 | 205 |
| 1 | 1 | 2 | 1 | 2 | 13 | 32 | 3 | 1 | 2 | 3 | 2 | 3 | 1 | 1 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 206 |
| 2 | 2 | 1 | 3 | 1 | 31 | 11 | 2 | 1 | 3 | 1 | 3 | 2 | 3 | 1 | 2 | 2 | 21 | 2 | 1 | 3 | 2 | 2 | 207 |
| 3 | 1 | 1 | 3 | 2 | 31 | 13 | 2 | 2 | 1 | 1 | 2 | 3 | 1 |  | 2 | 2 | 3 | 2 | 1 | 1 | 1 | 2 | 208 |
| 1 | 1 | 2 | 3 | 2 | 11 | 11 | 3 | 2 | 1 | 1 | 1 | 3 | 31 | 1 | 1 | 3 | 2 | 3 | 1 | 2 | 3 | 1 | 209 |
| 3 | 2 | 2 | 1 | 3 | 22 | 21 | 2 | 3 | 1 | 2 | 3 | 1 | 1 | 2 | 1 | 2 | 2 | 3 | 2 | 3 | 2 | 1 | 210 |
| 1 | 1 | 1 | 2 | 3 | 13 | 32 | 2 | 1 | 3 | 1 | 3 | 2 | 1 | 3 | 1 | 1 | 2 | 2 | 1 | 2 | 3 | 2 | 211 |
| 3 | 1 | 2 | 1 | 2 | 13 | 31 | 1 | 3 | 1 | 2 | 2 | 1 | 13 | 2 | 2 | 1 | 13 | 2 | 3 | 1 | 2 | 1 | 212 |
| 1 | 2 | 1 | 3 | 2 | 22 | 23 | 32 | 2 | 3 | 31 | 3 | 1 | 12 | 2 | 2 | 1 | 2 | 3 | 1 | 3 | 2 | 1 | 213 |
| 2 | 1 | 3 | 1 | 1 | 2 | 13 | 32 | 2 | 1 | 13 | 2 | 1 | 13 | 32 | 1 | 1 | 13 | 1 | 3 | 2 | 1 | 2 | 214 |
| 3 | 1 | 1 | 2 | 2 | 23 | 32 | 21 | 2 | 2 | 23 | 2 | 3 | 31 | 1 | 3 | 2 | 2 | 2 | 1 | 3 | 2 | 1 | 215 |
| 3 | 2 | 2 | 3 | 2 | 11 | 13 | 31 | 1 | 3 | 31 | 13 | 1 | 11 | 2 | 2 | 1 | 13 | 1 | 2 | 2 | 1 | 1 | 216 |
| 1 | 1 | 12 | 3 | 2 | 32 | 22 | 21 | 2 | 3 | 32 | 1 | 2 | 23 | 32 | 1 | 1 | 11 | 2 | 1 | 3 | 32 | 3 | 217 |
| 3 | 1 | 1 | 2 | 2 | 1 | 32 | 22 | 21 | 3 | 31 | 13 | 32 | 21 | 11 | 1 | 2 | 22 | 2 | 2 | 2 | 2 | 3 | 218 |
| 3 | 3 | 11 | 1 | 2 | 2 | 31 | 11 | 13 | 3 | 12 | 21 | 13 | 32 | 21 | 11 | 3 | 31 | 11 | 11 | 2 | 2 | 1 | 219 |
| 3 | 32 | 23 | 2 | 1 | 2 | 2 | 12 | 23 | 3 | 23 | 31 | 12 | 22 | 22 | 1 | 2 | 23 | 31 | 2 | 1 | 3 | 1 | 220 |
| 2 | 1 | 12 | 2 | 1 | 2 | 3 | 13 | 31 | 11 | 11 | 13 | 32 | 22 | 23 | 31 | 1 | 12 | 21 | 13 | 2 | 21 | 3 | 221 |
| 2 | 1 | 12 | 3 | 2 | 2 | 22 | 23 | 32 | 21 | 12 | 22 | 23 | 31 | 13 | 32 | 1 | 13 | 31 | 12 | 3 | 31 | 1 | 222 |
| 3 | 32 | 23 | 1 | 2 | 2 | 3 | 11 | 12 | 21 | 13 | 32 | 21 | 13 | 31 | 12 | 2 | 23 | 32 | 2 | 2 | 21 | 1 | 223 |
| 1 | 13 | 32 | 1 | 1 | 3 | 2 | 23 | 32 | 22 | 22 | 23 | 31 | 12 | 22 | 23 | 1 | 11 | 11 | 12 | 2 | 22 |  | 224 |
| 3 | 31 | 11 | 3 | 2 | 2 | 2 | 3 | 2 | 22 | 22 | 21 | 11 | 13 | 32 | 22 | 2 | 21 | 11 | 13 | 3 | 1 |  | 225 |
| 3 | 31 | 13 | 1 | 1 | 3 | 12 | 21 | 11 | 11 | 12 | 23 | 31 | 2 | 21 | 12 | 22 | 23 | 32 | 22 | 2 | 12 | 2 | 226 |
| 1 | 12 | 23 | 1 | 2 | 3 | 13 | 32 | 2 | 2 | 32 | 22 | 21 | 1 | 12 | 21 | 13 | 3 | 22 | 21 | 1 | 3 |  | 227 |
| 2 | 21 | 12 | 3 | 1 | 2 | 1 | 22 | 2 | 23 | 31 | 11 | 13 | 31 | 13 | 32 | 23 | 32 | 22 | 21 | 1 | 13 |  | 228 |
| 3 | 31 | 13 | 1 | 2 | 3 | 1 | 2 | 2 | 11 | 11 | 13 | 32 | 23 | 31 | 12 | 22 | 22 | 21 | 12 | 2 | 31 | 1 | 229 |
|  |  | 21 | 3 | 2 | 2 | 1 | 3 | 3 | 13 | 3 | 23 | 31 | 2 | 23 | 31 | 13 | 31 | 11 | 12 | 2 | 11 | 1 | 230 |
|  | 22 | 22 | 1 | 2 | 2 | 3 | 2 | 2 | 1 | 3 | 12 | 21 | 11 | 11 | 13 | 31 | 1 | 32 | 22 | 2 | 3 | 1 | 231 |
|  | 13 | 31 | 1 | 2 | 1 | 2 | 23 | 31 | 12 | 2 | 13 | 32 | 2 | 23 | 31 | 11 | 1 | 32 | 22 | 2 | 31 | 1 | 232 |
|  |  | 13 | 2 | 3 | 2 | 1 | 1 | 1 | 2 | 23 | 32 | 21 | 3 | 31 | 12 | 2 | 3 | 32 | 21 | 1 | 1 | 1 | 233 |
|  | 13 | 32 | 1 | 3 | 2 | 3 | 2 | 2 | 1 | 2 | 31 | 12 | 2 | 22 | 23 | 3 | 1 | 12 | 21 | 1 | 2 | 2 | 234 |
|  |  | 32 | 1 | 2 | 2 | 3 | 11 | 12 | 2 |  | 13 | 31 | 1 | 12 | 21 | 13 | 32 | 23 | 31 | 1 | 31 | 1 | 235 |
|  | 23 | 31 | 2 | 1 | 2 | 3 | 13 | 3 | 1 | 2 | 13 | 31 | 11 | 3 | 32 | 2 | 2 | 2 | 11 | 1 | 2 | 3 | 236 |
|  | 31 | 11 | 3 | 1 | 1 | 3 | 2 | 1 | 1 | 3 | 21 | 12 | 2 | 11 | 11 | 1 | 2 | 2 | 11 | 1 | 12 | 2 | 237 |
|  | 22 | 22 | 1 | 1 | 3 | 2 | 3 | 2 | 3 | 1 | 21 | 11 | 1 | 31 | 11 | 1 | 3 | 3 | 12 | 2 | 13 | 3 | 238 |
|  | 21 | 12 | 2 | 3 | 2 | 2 | 3 | 1 | 1 | 2 | 32 | 2 | 3 | 22 | 22 | 2 | 1 | 11 | 13 | 3 | 1 | 3 | 239 |
|  |  |  |  |  |  |  |  | 1 |  |  |  |  | 12 | 23 | 31 | 12 | 2 | 21 | 12 | 2 | 2 | 3 | 240 |

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Table IA: Numeric sequences corresponding to nucleotide base patterns of a set of oligonucleotides

Numeric Pattern


Table IA: Numeric sequences corresponding to nucleotide base patterns of a set of oligonucleotides Numeric Pattern


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Sequence
Identifier

Table IA: Numeric sequences corresponding to nucleotide
base patterns of a set of oligonucleotides
Numeric Pattern Identifier


Table IA: Numeric sequences corresponding to nucleotide base patterns of a set of oligonucleotides

| 3 |  |  | 1 |  |  | 2 | 3 | 1 | 2 | 2 | 2 | 1 | 3 | 1 | 3 | 2 | 1 | 1 | 2 | 1 |  | 3 |  | 633 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 1 | 1 | 2 | 1 | 1 | 3 | 1 | 1 | 2 | 1 | 3 | 1 | 3 | 1 | 3 | 1 | 3 | 1 | 1 | 1 | 3 | 2 | 2 | 634 |
| 3 | 2 | 2 | 3 | 2 | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 2 | 1 | 3 | 1 | 3 | 1 | 1 | 1 | 2 | 2 | 1 | 3 | 635 |
| 1 | 3 | 2 | 3 | 1 | 2 | 2 | 2 | 1 | 3 | 1 | 2 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 2 | 636 |
| 1 | 3 | 1 | 3 | 2 | 1 | 2 | 1 | 3 | 2 | 2 | 2 | 1 | 3 | 1 | 2 | 2 | 2 | 1 | 2 | 3 | 2 | 1 | 3 | 637 |
| 1 | 2 | 3 | 1 | 2 | 2 | 1 | 3 | 1 | 2 | 1 | 3 | 2 | 3 | 1 | 1 | 1 | 2 | 2 | 3 | 2 | 2 | 1 | 3 | 638 |
| 1 | 2 | 3 | 1 | 1 | 1 | 2 | 3 | 2 | 1 | 2 | 2 | 1 | 3 | 2 | 2 | 2 | 1 | 3 | 1 | 3 | 2 | 2 | 3 | 639 |
| 1 | 2 | 1 | 2 | 2 | 3 | 1 | 3 | 2 | 3 | 1 | 3 | 1 | 3 | 2 | 2 | 1 | 2 | 2 | 3 | 2 | 2 | 1 | 1 | 640 |
| 1 | 3 | 1 | 2 | 3 | 2 | 3 | 2 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 2 | 1 | 1 | 3 | 1 | 1 | 3 | 2 | 2 | 641 |
| 2 | 1 | 1 | 2 | 3 | 2 | 3 | 2 | 2 | 3 | 1 | 2 | 1 | 3 | 1 | 1 | 2 | 1 | 3 | 1 | 3 | 1 | 2 | 1 | 642 |
| 1 | 1 | 1 | 2 | 2 | 1 | 3 | 2 | 2 | 3 | 1 | 1 | 1 | 3 | 2 | 1 | 2 | 3 | 1 | 3 | 1 | 1 | 1 | 3 | 643 |
| 2 | 2 | 2 | 1 | 3 | 1 | 3 | 2 | 2 | 3 | 1 | 1 | 3 | 1 | 1 | 1 | 2 | 3 | 2 | 2 | 1 | 1 | 2 | 3 | 644 |
| 3 | 1 | 2 | 2 | 3 | 2 | 2 | 3 | 1 | 2 | 2 | 1 | 2 | 2 | 3 | 1 | 2 | 3 | 1 | 1 | 2 | 2 | 2 | 3 | 645 |
| 2 | 3 | 2 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 3 | 1 | 1 | 2 | 2 | 3 | 1 | 1 | 3 | 1 | 1 | 2 | 2 | 1 | 646 |
| 1 | 2 | 2 | 1 | 1 | 3 | 2 | 1 | 1 | 3 | 1 | 1 | 2 | 2 | 3 | 1 | 3 | 1 | 3 | 2 | 2 | 2 | 3 | 1 | 647 |
| 3 | 2 | 1 | 2 | 3 | 2 | 2 | 3 | 2 | 1 | 1 | 2 | 3 | 2 | 1 | 2 | 2 | 1 | 1 | 3 | 1 | 1 | 1 | 3 | 648 |
| 2 | 1 | 3 | 2 | 2 | 3 | 2 | 3 | 1 | 2 | 2 | 2 | 1 | 2 | 3 | 2 | 1 | 1 | 2 | 3 | 1 | 2 | 2 |  | 649 |
| 2 | 3 | 1 | 2 | 1 | 1 | 2 | 3 | 1 | 1 | 1 | 3 | 2 | 2 | 2 | 1 | 2 | 1 | 3 | 1 | 3 | 1 | 3 | 1 | 650 |
| 3 | 2 | 1 | 1 | 3 | 1 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 2 | 1 | 2 | 3 | 1 | 2 | 1 | 1 | 3 | 1 | 2 | 651 |
| 2 | 2 | 3 | 1 | 1 | 2 | 2 | 1 | 1 | 3 | 1 | 3 | 2 | 1 | 1 | 3 | 1 | 2 | 3 | 2 | 2 | 2 | 1 | 3 | 652 |
| 1 | 1 | 3 | 32 | 3 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 1 | 3 | 1 | 3 | 2 | 1 | 1 | 1 | 3 | 1 | 2 | 1 | 653 |
| 1 | 3 | 1 | 3 | 1 | 3 | 1 | 2 | 1 | 1 | 1 | 3 | 2 | 1 | 2 | 1 | 3 | 1 | 1 | 3 | 2 | 2 | 1 | 1 | 654 |
| 1 | 12 | 2 | 1 | 2 | 3 | 1 | 1 | 2 | 1 | 3 | 2 | 2 | 1. | 3 | 1 | 1 | 1 | 3 | 1 | 3 | 1 | 3 | 2 | 655 |
| 2 | 2 | 3 | 32 | 2 | 3 | 1 | 2 | 1 | 2 | 2 | 1 | 3 | 1 | 3 | 1 | 1 | 2 | 3 | 2 | 3 | 2 | 2 | 2 | 656 |
| 2 | 2 | 2 | 21 | 2 | 2 | 3 | 1 | 3 | 1 | 3 | 2 | 2 | 2 | 3 | 2 | 2 | 1 | 2 | 2 | 2 | 3 | 2 | 3 | 657 |
| 1 | 1 | 2 | 23 | 2 | 2 | 1 | 1 | 3 | 1 | 1 | 3 | 2 | 2 | 3 | 1 | 2 | 2 | 1 | 2 | 2 | 3 | 1 | 2 | 658 |
| 3 | 31 | 3 | 31 | 1 | 1 | 2 | 3 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 2 | 2 | 3 | 1 | 2 | 1 | 1 | 2 | 659 |
| 3 | 3 | 2 | 21 | 1 | 3 | 2 | 1 | 2 | 2 | 1 | 3 | 2 | 1 | 2 | 3 | 1 | 3 | 2 | 3 | 2 | 1 | 1 | 2 | 660 |
| 2 | 22 | 2 | 23 | 1 | 2 | 2 | 2 | 1 | 1 | 3 | 1 | 3 | 2 | 3 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 2 | 1 | 661 |
| 1 | 11 | 13 | 32 | 2 | 1 | 3 | 2 | 1 | 1 | 1 | 2 | 1 | 3 | 1 | 3 | 2 | 1 | 3 | 1 | 1 | 1 | 3 | 1 | 662 |
| 3 | 32 | 1 | 11 | 1 | 3 | 2 | 1 | 2 | 3 | 1 | 1 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 1 | 1 | 2 | 3 | 2 | 663 |
| 2 | 21 | 11 | 12 | 21 | 1 | 3 | 2 | 3 | 2 | 3 | 2 | 2 | 3 | 2 | 3 | 1 | 1 | 2 | 3 | 2 | 1 | 1 | 2 | 664 |
|  | 11 | 11 | 13 | 31 | 2 | 2 | 2 | 1 | 3 | 1 | 3 | 2 | 1 | 3 | 1 | 1 | 1 | 3 | 1 | 3 | 1 | 1 | 2 | 665 |
|  | 22 | 21 | 13 | 32 | 2 | 2 | 3 | 1 | 3 | 2 | 2 | 3 | 1 | 1 | 1 | 2 | 1 | 3 | 2 | 1 | 1 | 1 | 3 | 666 |
|  | 21 | 11 | 12 | 21 | 3 | 2 | 21 | 2 | 3 | 1 | 3 | 2 | 1 | 1 | 3 | 1 | 2 | 2 | 3 | 1 | 1 | 1 | 3 | 667 |
|  | 31 | 11 | 13 | 3 | 2 | 2 | 21 | 13 | 1 | 2 | 2 | 3 | 1 | 2 | 3 | 2 | 2 | 1 | 3 | 2 | 2 | 1 | 1 | 668 |
|  | 21 | 11 | 11 | 13 | 1 | 13 | 31 | 13 | 1 | 1 | 3 | 2 | 2 | 1 | 3 | 3 | 1 | 1 | 2 | 1 | 3 | 1 |  | 669 |
|  | 21 | 11 | 13 | 32 | 1 | 12 | 23 | 1 | 3 | 1 | 1 | 1 | 2 | 3 | 1 | 2 | 3 | 2 | 3 | 2 | 2 | 1 |  | 670 |
|  | 31 | 13 | 32 | 22 | 2 | 3 | 32 | 22 | 2 | 3 | 2 | 2 | 1 | 3 | 2 | 2 | 1 | 2 | 2 | 3 | 1 | 2 |  | 671 |
|  | 11 | 13 | 32 | 21 | 1 | 1 | 13 | 31 | 1 | 1 | 2 | 3 | 2 | 2 | 1 | 1 | 3 | 1 | 3 | 2 | 1 | 3 |  | 672 |
|  | 12 | 23 | 31 | 13 | 31 | 11 | 12 | 22 | 2 | 2 | 2 | 3 | 2 | 2 | 3 | 1 | 1 | 1 | 2 | 3 | 2 | 1 |  | 673 |
|  | 22 | 21 | 13 | 31 | 12 | 22 | 21 | 13 | 31 | 3 | 2 | 1 | 3 | 32 | 1 | 13 | 1 | 1 | 13 | 1 | 1 | 12 |  | 674 |
|  | 21 | 13 | 32 | 23 | 31 | 12 | 23 | 31 | 11 | 13 | 1 | 1 | 3 | 32 | 2 | 2 | 3 | 1 | 1 | 1 | 2 | 22 | 1 | 675 |
|  | 21 | 11 | 12 | 23 | 32 | 21 | 13 | 32 | 21 | 11 | 2 | 3 | 2 | 23 | 1 | 1 | 3 | 1 | 2 | 1 | 1 | 13 |  | 676 |
|  | 22 | 23 | 31 | 13 | 31 | 11 | 11 | 13 | 31 | 11 | 2 | 1 | 1 | 13 | 2 | 21 | 1 | 2 | 23 | 2 |  | 11 |  | 677 |
|  | 21 | 11 | 12 | 23 | 31 | 13 | 32 | 23 | 31 | 13 | 1 | 12 | 2 | 21 | 2 | 21 | 13 | 1 | 2 | 2 | 3 | 32 |  | 678 |
|  | 2 | 21 | 12 | 21 | 11 | 13 | 31 | 11 | 11 | 12 | 3 |  | 3 | 32 | 3 | 32 | 2 | 2 | 3 | 1 |  | 22 |  | 679 |
|  | 2 | 23 | 31 | 11 | 12 | 23 | 32 | 23 | 32 | 22 | 2 | 1 | 2 | 23 | 1 | 11 | 1 | 1 | 2 | 1 |  |  |  | 680 |
|  | 1 | 11 | 11 | 13 | 32 | 22 | 1 | 12 | 22 | 21 | 13 | 3 | 1 | 13 | 32 | 22 | 21 | 1 | 1 | 3 |  | 2 |  | 681 |
|  | 21 | 13 | 31 | 11 | 12 | 22 | 3 | 32 | 23 | 32 | 22 | 2 | 2 | 31 | 12 | 21 | 11 | 3 | 2 | 3 |  |  |  | 682 |
|  |  | 31 | 12 | 22 | 22 | 2 | 3 | 31 | 12 | 22 | 23 | 31 | 13 | 31 | 13 | 32 | 22 | 1 | 1 | 1 | 12 | 2 | 3 | 683 |
|  |  | 22 | 21 | 12 | 22 | 2 | 31 | 3 | 32 | 22 | 22 | 23 | 31 | 11 | 2 | 2 | 32 | 2 | 3 | 31 |  | 21 |  | 684 |
|  |  | 21 | 13 | 32 | 21 | 13 | 32 | 2 | 21 | 12 | 23 | 32 | 22 | 22 | 23 | 31 | 12 | 2 | 2 | 21 | 1 | 3 | 2 | 685 |
|  |  | 21 | 13 | 31 | 11 | 1 | 31 | 11 | 3 | 31 | 11 | 12 | 21 | 11 | 1 | 13 | 32 | 2 | 21 | 13 | 31 | 1 | 3 | 686 |
|  |  | 12 | 23 | 32 | 22 | 2 | 31 | 11 | 1 | 13 | 32 | 21 | 11 | 12 | 23 | 31 | 11 | 12 | 22 | 2 | 2 | 3 |  | 687 |
|  |  | 13 | 31 | 12 | 22 | 23 | 31 | 12 | 21 | 13 | 32 | 21 | 13 | 31 | 11 | 11 | 12 | 23 | 31 | 2 | 2 | 11 | 1 | 688 |

# Table IA: <br> Numeric sequences corresponding to nucleotide <br> base patterns of a set of oligonucleotides 

Numeric Pattern
Sequence
Identifier


Table IA: Numeric sequences corresponding to nucleotide
base patterns of a set of oligonucleotides
Numeric Pattern


## Table IA:

Numeric sequences corresponding to nucleotide base patterns of a set of oligonucleotides Numeric Pattern

Sequence Identifier


Table IA: Numeric sequences corresponding to nucleotide base patterns of a set of oligonucleotides



Table IA: Numeric sequences corresponding to nucleotide base patterns of a set of oligonucleotides


Table IA: Numeric sequences corresponding to nucleotide base patterns of a set of oligonucleotides Numeric Pattern


Table IA: Numeric sequences corresponding to nucleotide base patterns of a set of oligonucleotides Numeric Pattern


Table IA: Numeric sequences corresponding to nucleotide base patterns of a set of oligonucleotides Numeric Pattern

Sequence Identifier


In Table IA, each of the numerals 1 to 3 (numeric identifiers) represents a nucleotide base and the pattern of numerals 1 to 3 of the sequences in the above list corresponds to the pattern of nucleotide bases present in the oligonucleotides of Table $I$, which oligonucleotides have been found to be non-cross-hybridizing, as described further in the detailed examples. Each nucleotide base is selected from the group of nucleotide bases consisting of $A, C, G$, and $T / U$. A particularly preferred embodiment of the invention, in which a specific base is assigned to each numeric identifier is shown in Table $I$, below.

In one broad aspect, the invention is a composition comprising molecules for use as tags or tag complements wherein each molecule comprises an oligonucleotide selected from a set of oligonucleotides based on a group of sequences as specified by numeric identifiers set out in Table IA. In the sequences, each of 1 to 3 is a nucleotide base
selected to be different from the others of 1 to 3 with the proviso that up to three nucleotide bases of each sequence can be substituted with any nucleotide base provided that:
for any pair of sequences of the set:
M1 s 15, M2 s 12, M3 s 19, M4 s 15, and M5 s 18, where:
M1 is the maximum number of matches for any alignment in which there are no internal indels;

M2 is the maximum length of a block of matches for any alignment;
M3 is the maximum number of matches for any alignment having a maximum score;
M4 is the maximum sum of the lengths of the longest two blocks of matches for any alignment of maximum score; and

M5 is the maximum sum of the lengths of all the blocks of matches having a length of at least 3, for any alignment of maximum score; wherein:
the score of an alignment is determined according to the equation (A $x \mathrm{~m})-(\mathrm{B} \times \mathrm{mm})-(\mathrm{C} \times(\mathrm{og}+\mathrm{eg}))-(\mathrm{D} \times \mathrm{eg}))$, wherein:
for each of (i) to (iv):
(i) $m=6, m m=6, \circ g=0$ and $e g=5$,
(ii) $m=6, \mathrm{~mm}=6, \mathrm{og}=5$ and $\mathrm{eg}=1$,
(iii) $m=6, m m=2, o g=5$ and $\mathrm{eg}=1$, and
(iv) $m=6, m m=6, o g=6$ and $e g=0$,
$A$ is the total number of matched pairs of bases in the alignment;
$B$ is the total number of internal mismatched pairs in the alignment;
$C$ is the total number of internal gaps in the alignment; and $D$ is the total number of internal indels in the alignment minus the total number of internal gaps in the alignment; and wherein the maximum score is determined separately for each of (i), (ii), (iii) and (iv).

An explanation of the meaning of the parameters set out above is given in the section describing detailed embodiments.

In another broad aspect, the invention is a composition containing molecules for use as tags or tag complements wherein each molecule comprises an oligonucleotide selected from a set of oligonucleotides based on a group of sequences as set out in Table IA wherein each of 1 to 3 is a nucleotide base selected to be different from the others of 1 to 3 with the proviso that up to three nucleotide bases of each sequence can be substituted with any nucleotide base provided that:
for any pair of sequences of the set:

M1 s 18, M2 s $16, M 3 \leq 20, M 4 \leq 17$, and $M 5 \leq 19$, where:
MI is the maximum number of matches for any alignment in which there are no internal indels;

M2 is the maximum length of a block of matches for any alignment; M3 is the maximum number of matches for any alignment having a maximum score;

M4 is the maximum sum of the lengths of the longest two blocks of matches for any alignment of maximum score; and M5 is the maximum sum of the lengths of all the blocks of matches having a length of at least 3 , for any alignment of maximum score; wherein
the score of an alignment is determined according to the equation ( $A$
$x \mathrm{~m})-(\mathrm{B} \times \mathrm{mm})-(\mathrm{C} \times(\mathrm{g}+\mathrm{eg}))-(\mathrm{D} \times \mathrm{eg}))$, wherein:
for each of (i) to (iv):
(i) $m=6, m m=6, o g=0$ and $\mathrm{eg}=6$,
(ii) $m=6, m m=6, o g=5$ and $e g=1$,
(iii) $m=6, \mathrm{~mm}=2, o g=5$ and $e g=1$, and
(iv) $m=6, m m=6, o g=6$ and $e g=0$,

A is the total number of matched pairs of bases in the alignment; $B$ is the total number of internal mismatched pairs in the alignment;
$C$ is the total number of internal gaps in the alignment; and $D$ is the total number of internal indels in the alignment minus the total number of internal gaps in the alignment; and wherein the maximum score is determined separately for each of (i), (ii), (iii) and (iv).

In another broad aspect, the invention is a composition comprising molecules for use as tags or tag complements wherein each molecule comprises an oligonucleotide selected from a set of oligonucleotides based on a group of sequences set out in Table IA wherein each of 1 to 3 is a nucleotide base selected to be different from the others of 1 to 3 with the proviso that up to three nucleotide bases of each sequence can be substituted with any nucleotide base provided that:
for any pair of sequences of the set:
M1 s 18, M2 s $16, \mathrm{M} 3 \leq 20, \mathrm{M} 4 \leq 17$, and M5 s 19 , where:
M1 is the maximum number of matches for any alignment in which there are no internal indels;

M2 is the maximum length of a block of matches for any alignment; M3 is the maximum number of matches for any alignment having a maximum
score;
M4 is the maximum sum of the lengths of the longest two blocks of matches for any alignment of maximum score; and M5 is the maximum sum of the lengths of all the blocks of matches having a length of at least 3, for any alignment of maximum score, wherein:
the score of an alignment is determined according to the equation 3 A - B - 3C - D, wherein:
$A$ is the total number of matched pairs of bases in the alignment; $B$ is the total number of internal mismatched pairs in the alignment;
$C$ is the total number of internal gaps in the alignment; and $D$ is the total number of internal indels in the alignment minus the total number of internal gaps in the alignment; and In preferred aspects, the invention provides a composition in which, for the group of 24 mer sequences in which $1=A, 2=T$ and $3=G$, under a defined set of conditions in which the maximum degree of hybridization between a sequence and any complement of a different sequence of the group of 24 mer sequences does not exceed $30 \%$ of the degree of hybridization between said sequence and its complement, for all said oligonucleotides of the composition, the maximum degree of hybridization between an oligonucleotide and a complement of any other oligonucleotide of the composition does not exceed $50 \%$ of the degree of hybridization of the oligonucleotide and its complement.

More preferably, the maximum degree of hybridization between a sequence and any complement of a different sequence does not exceed $30 \%$ of the degree of hybridization between said sequence and its complement, the degree of hybridization between each sequence and its complement varies by a factor of between 1 and up to 10 , more preferably between 1 and up to 9 , more preferably between 1 and up to 8 , more preferably between 1 and up to 7 , more preferably between 1 and up to 6 , and more preferably between 1 and up to 5 .

It is also preferred that the maximum degree of hybridization between a sequence and any complement of a different sequence does not exceed 25\%, more preferably does not exceed $20 \%$, more preferably does not exceed 15\%, more preferably does not exceed 10\%, more preferably does not exceed $5 \%$.

Even more preferably, the above-referenced defined set of conditions results in a level of hybridization that is the same as the
level of hybridization obtained when hybridization conditions include $0.2 \mathrm{M} \mathrm{NaCl}, 0.1 \mathrm{M}$ Tris, $0.08 \%$ Triton $\mathrm{X}-100, \mathrm{pH} 8.0$ at $37^{\circ} \mathrm{C}$.

In the composition, the defined set of conditions can include the group of 24 mer sequences being covalently linked to beads.

In a particular preferred aspect, for the group of 24 mers the maximum degree of hybridization between a sequence and any complement of a different sequence does not exceed $15 \%$ of the degree of hybridization between said sequence and its complement and the degree of hybridization between each sequence and its complement varies by a factor of between 1 and up to 9 , and for all oligonucleotides of the set, the maximum degree of hybridization between an oligonucleotide and a complement of any other oligonucleotide of the set does not exceed $20 \%$ of the degree of hybridization of the oligonucleotide and its complement.

It is possible that each 1 is one of $A, T / U, G$ and $C$; each 2 is one of $A, T / U, G$ and $C$; and each 3 is one of $A, T / U, G$ and $C$; and each of 1,2 and 3 is selected so as to be different from all of the others of 1,2 and 3. More preferably, 1 is $A$ or $T / U, 2$ is $A$ or $T / U$ and 3 is $G$ or C. Even more preferably, 1 is $A, 2$ is $T / U$, and 3 is $G$.

In certain preferred composition, each of the oligonucleotides is from twenty-two to twenty-six bases in length, or from twenty-three to twenty-five, and preferably, each oligonucleotide is of the same length as every other said oligonucleotide.

In a particularly preferred embodiment, each oligonucleotide is twenty-four bases in length.

It is preferred that no oligonucleotide contains more than four contiguous bases that are identical to each other.

It is also preferred that the number of G's in each
oligonucleotide does not exceed $L / 4$ where $L$ is the number of bases in said sequence.

For reasons described below, the number of G's in each said oligonucleotide is preferred not to vary from the average number of G's in all of the oligonucleotides by more than one. Even more preferably, the number of $G^{\prime} s$ in each said oligonucleotide is the same as every other said oligonucleotide. In the embodiment disclosed below in which oligonucleotides were tested, the sequence of each was twenty-four bases in length and each oligonucleotide contained 6 G's.

It is also preferred that, for each nucleotide, there is at most six bases other than $G$ between every pair of neighboring pairs of $G^{\prime} s$.

Also, it is preferred that, at the 5'-end of each oligonucleotide at least one of the first, second, third, fourth, fifth, sixth and
seventh bases of the sequence of the oligonculeotide is a G. Similarly, it is preferred, at the $3^{\prime}$-end of each oligonucleotide that at least one of the first, second, third, fourth, fifth, sixth and seventh bases of the sequence of the oligonucleotide is a G.

It is possible to have sequence compositions that include one hundred and sixty said molecules, or that include one hundred and seventy said molecules, or that include one hundred and eighty said molecules, or that include one hundred and ninety said molecules, or that include two hundred said molecules, or that include two hundred and twenty said molecules, or that include two hundred and forty said molecules, or that include two hundred and sixty said molecules, or that include two hundred and eighty said molecules, or that include three hundred said molecules, or that include four hundred said molecules, or that include five hundred said molecules, or that include six hundred said molecules, or that include seven hundred said molecules, or that include eight hundred said molecules, or that include nine hundred said molecules, or that include one thousand said molecules.

It is possible, in certain applications, for each molecule to be linked to a solid phase support so as to be distinguishable from a mixture containing other of the molecules by hybridization to its complement. Such a molecule can be linked to a defined location on a solid phase support such that the defined location for each molecule is different than the defined location for different others of the molecules.

In certain embodiments, each solid phase support is a microparticle and each said molecule is covalently linked to a different microparticle than each other different said molecule.

In another broad aspect, the invention is a composition comprising a set of 150 molecules for use as tags or tag complements wherein each molecule includes an oligonucleotide having a sequence of at least sixteen nucleotide bases wherein for any pair of sequences of the set:

M1 > 19/24 $\times \mathrm{L} 1, \mathrm{M} 2>17 / 24 \times \mathrm{Ll}, \mathrm{M} 3>21 / 24 \times \mathrm{Ll}, \mathrm{M} 4{ }^{\circ}>18 / 24 \times \mathrm{Ll}, \mathrm{M} 5>$ $20 / 24 \times \mathrm{Ll}$, where Ll is the length of the shortest sequence of the pair, where:
M1 is the maximum number of matches for any alignment of the pair of sequences in which there are no internal indels;
M2 is the maximum length of a block of matches for any alignment of the pair of sequences;
M3 is the maximum number of matches for any alignment of the pair of sequences having a maximum score;

M4 is the maximum sum of the lengths of the longest two blocks of matches for any alignment of the pair of sequences of maximum score; and

M5 is the maximum sum of the lengths of all the blocks of matches having a length of at least 3 , for any alignment of the pair of sequences of maximum score, wherein:
the score of an alignment is determined according to the equation ( $A$ $x m)-(B \times m m)-(C \times(o g+e g))-(D \times e g))$, wherein:
for each of (i) to (iv):
(i) $\quad m=6, \mathrm{~mm}=6, \circ \mathrm{og}=0$ and $\mathrm{eg}=6$,
(ii) $m=6, m m=6, o g=5$ and $e g=1$,
(iii) $m=6, m m=2, o g=5$ and $e g=1$, and
(iv) $m=6, m m=6, o g=6$ and $e g=0$,

A is the total number of matched pairs of bases in the alignment; $B$ is the total number of internal mismatched pairs in the alignment;
$C$ is the total number of internal gaps in the alignment; and $D$ is the total number of internal indels in the alignment minus the total number of internal gaps in the alignment; and wherein the maximum score is determined separately for each of (i), (ii), (iii) and (iv).

In yet another broad aspect, the invention is a composition that includes a set of 150 molecules for use as tags or tag complements wherein each molecule has an oligonucleotide having a sequence of at least sixteen nucleotide bases wherein for any pair of sequences of the set:

M1 s 18, M2 s $16, M 3 \leq 20, M 4 \leq 17$, and M5 $\leq 19$, where:
M1 is the maximum number of matches for any alignment of the pair of sequences in which there are no internal indels;

M2 is the maximum length of a block of matches for any alignment of the pair of sequences;

M3 is the maximum number of matches for any alignment of the pair of sequences having a maximum score;
M4 is the maximum sum of the lengths of the longest two blocks of matches for any alignment of the pair of sequences of maximum score; and

M5 is the maximum sum of the lengths of all the blocks of matches having a length of at least 3 , for any alignment of the pair of sequences of maximum score, wherein:
the score of a said alignment is determined according to the equation
$3 A-B-3 C-D$, wherein:
A is the total number of matched pairs of bases in the alignment;
$B$ is the total number of internal mismatched pairs in the alignment;
$C$ is the total number of internal gaps in the alignment; and
$D$ is the total number of internal indels in the alignment minus the total number of internal gaps in the alignment.
In certain embodiments of the invention, each sequence of a composition has up to fifty bases. More preferably, however, each sequence is between sixteen and forty bases in length, or between sixteen and thirty-five bases in length, or between eighteen and thirty bases in length, or between twenty and twenty-eight bases in length, or between twenty-one and twenty-seven bases in length, or between twentytwo and twenty-six bases in length

Often, each sequence is of the same length as every other said sequence. In particular embodiments disclosed herein, each sequence is twenty-four bases in length.

Again, it can be preferred that no sequence contains more than four contiguous bases that are identical to each other, etc., as described above.

In certain preferred embodiments, the composition is such that, under a defined set of conditions, the maximum degree of hybridization between an oligonucleotide and any complement of a different oligonucleotide of the composition does not exceed about $30 \%$ of the degree of hybridization between said oligonucleotide and its complement, more preferably 20\%, more preferably 15\%, more preferably 10\%, more preferably 6\%.

Preferably, the set of conditions results in a level of hybridization that is the same as the level of hybridization obtained when hybridization conditions include $0.2 \mathrm{M} \mathrm{NaCl}, 0.1 \mathrm{M} \mathrm{Tris} 0.08 \$, Triton $\mathrm{X}-100, \mathrm{pH} 8.0$ at $37^{\circ} \mathrm{C}$, and the oligonucleotides are covalently linked to microparticles. Of course it is possible that these specific conditions be used for determining the level of hybridization.

It is also preferred that under such a defined set of conditions, the degree of hybridization between each oligonucleotide and its complement varies by a factor of between 1 and up to 8 , more preferably up to 7, more preferably up to 6, more preferably up to 5. In a particular disclosed embodiment, the observed variance in the degree of hybridization was a factor of only 5.3, i.e., the degree of
hybridization between each oligonucleotide and its complement varied by a factor of between 1 and 5.6.

In certain preferred embodiments, under the defined set of conditions, the maximum degree of hybridization between a said oligonucleotide and any complement of a different oligonucleotide of the composition does not exceed about 15\%, more preferably 10\%, more preferably 6\%.

In one preferred embodiment, the set of conditions results in a level of hybridization that is the same as the level of hybridization obtained when hybridization conditions include $0.2 \mathrm{M} \mathrm{NaCl}, 0.1 \mathrm{M} \mathrm{Tris}$, $0.08 \%$ Triton $\mathrm{X}-100, \mathrm{pH} 8.0$ at $37^{\circ} \mathrm{C}$, and the oligonucleotides are covalently linked to microparticles.

Also, under the defined set of conditions, it is preferred that the degree of hybridization between each oligonucleotide and its complement varies by a factor of between 1 and up to 8 , more preferably up to 7, more preferably up to 6, more preferably up to 5.

Any composition of the invention can include one hundred and sixty of the oligonucleotide molecules, or one hundred and seventy of the oligonucleotide molecules, or one hundred and eighty of the oligonucleotide molecules, or one hundred and ninety of the oligonucleotide molecules, or two hundred of the oligonucleotide molecules, or two hundred and twenty of the oligonucleotide molecules, or two hundred and forty of the oligonucleotide molecules, or two hundred and sixty of the oligonucleotide molecules, or two hundred and eighty of the oligonucleotide molecules, or three hundred of the oligonucleotide molecules, or four hundred of the oligonucleotide molecules, or five hundred of the oligonucleotide molecules, or six hundred of the oligonucleotide molecules, or seven hundred of the oligonucleotide molecules, or eight hundred of the oligonucleotide molecules, or nine hundred of the oligonucleotide molecules, or one thousand or more of the oligonucleotide molecules

A composition of the invention can be a family of tags, or it can be a family of tag complements.

An oligonucleotide molecule belonging to a family of molecules of the invention can have incorporated thereinto one more analogues of nucleotide bases, preference being given those that undergo normal Watson-Crick base pairing.

The invention includes kits for sorting and identifying polynucleotides. Such a kit can include one or more solid phase supports each having one or more spatially discrete regions, each such
region having a uniform population of substantially identical tag complements covalently attached. The tag complements are made up of a set of oligonucleotides of the invention.

The one or more solid phase supports can be a planar substrate in which the one or more spatially discrete regions is a plurality of spatially addressable regions.

The tag complements can also be coupled to microparticles. Microparticles preferably each have a diameter in the range of from 5 to $40 \mu \mathrm{~m}$.

Such a kit preferably includes microparticles that are spectrophotometrically unique, and therefore distinguisable from each other according to conventional laboratory techniques. Of course for such kits to work, each type of microparticle would generally have only one tag complement associated with it, and usually there would be a different oligonucleotide tag complement associated with (attached to) each type of microparticle.

The invention includes methods of using families of oligonucleotides of the invention.

One such method is of analyzing a biological sample containing a biological sequence for the presence of a mutation or polymorphism at a locus of the nucleic acid. The method includes:
(A) amplifying the nucleic acid molecule in the presence of a first primer having a 5'-sequence having the sequence of a tag complementary to the sequence of a tag complement belonging to a family of tag complements of the invention to form an amplified molecule with a $5^{\prime}$-end with a sequence complementary to the sequence of the tag; extending the amplified molecule in the presence of a polymerase and a second primer having $5^{\prime}$-end complementary the $3^{\prime}$-end of the amplified sequence, with the $3^{\prime}$-end of the second primer extending to immediately adjacent said locus, in the presence of a plurality of nucleoside triphosphate derivatives each of which is: (i) capable of incorporation during transciption by the polymerase onto the $3^{\prime}$-end of a growing nucleotide strand; (ii) causes termination of polymerization; and (iii) capable of differential detection, one from the other, wherein there is a said derivative complementary to each possible nucleotide present at said locus of the amplified sequence;
(C) specifically hybridizing the second primer to a tag complement having the tag complement sequence of (A); and
(D) detecting the nucleotide derivative incorporated into the second primer in (B) so as to identify the base located at the locus of the nucleic
acid.
In another method of the invention, a biological sample containing a plurality of nucleic acid molecules is analyzed for the presence of a mutation or polymorphism at a locus of each nucleic acid molecule, for each nucleic acid molecule. This method includes steps of:
(A) amplifying the nucleic acid molecule in the presence of a first primer having a 5'-sequence having the sequence of a tag complementary to the seguence of a tag complement belonging to a family of tag complements of the invention to form an amplified molecule with a 5'-end with a sequence complementary to the sequence of the tag;
(B) extending the amplified molecule in the presence of a polymerase and a second primer having $5^{\prime}$-end complementary the $3^{\prime}$-end of the amplified sequence, the $3^{\prime}$-end of the second primer extending to immediately adjacent said locus, in the presence of a plurality of nucleoside triphosphate derivatives each of which is: (i) capable of incorporation during transciption by the polymerase onto the $3^{\prime}$-end of a growing nucleotide strand; (ii) causes termination of polymerization; and (iii) capable of differential detection, one from the other, wherein there is a said derivative complementary to each possible nucleotide present at said locus of the amplified molecule;
(C) specifically hybridizing the second primer to a tag complement having the tag complement sequence of (A); and
(D) detecting the nucleotide derivative incorporated into the second primer in (B) so as to identify the base located at the locus of the nucleic acid;
wherein each tag of ( $A$ ) is unique for each nucleic acid molecule and steps (A) and (B) are carried out with said nucleic molecules in the presence of each other

Another method includes analyzing a biological sample that contains a plurality of double stranded complementary nucleic acid molecules for the presence of a mutation or polymorphism at a locus of each nucleic acid molecule, for each nucleic acid molecule. The method includes steps of:
(A) amplifying the double stranded molecule in the presence of a pair of first primers, each primer having an identical 5'-sequence having the sequence of a tag complementary to the sequence of a tag complement belonging to a family of tag complements of the invention to form amplified molecules with 5 '-ends with a sequence complementary to the sequence of the tag;
(B) extending the amplified molecules in the presence of a polymerase and a
pair of second primers each second primer having a $5^{\prime}$-end complementary a $3^{\prime}$-end of the amplified sequence, the $3^{\prime}$-end of each said second primer extending to immediately adjacent said locus, in the presence of a plurality of nucleoside triphosphate derivatives each of which is: (i) capable of incorporation during transciption by the polymerase onto the $3^{\prime}$-end of a growing nucleotide strand; (ii) causes termination of polymerization; and (iii) capable of differential detection, one from the other;
(C) specifically hybridizing each of the second primers to a tag complement having the tag complement sequence of (A); and (D) detecting the nucleotide derivative incorporated into the second primers in (B) so as to identify the base located at said locus; wherein the sequence of each tag of (A) is unique for each nucleic acid molecule and steps (A) and (B) are carried out with said nucleic molecules in the presence of each other.

In yet another aspect, the invention is a method of analyzing a biological sample containing a plurality of nucleic acid molecules for the presence of a mutation or polymorphism at a locus of each nucleic acid molecule, for each nucleic acid molecule, the method including steps of:
(a) hybridizing the molecule and a primer, the primer having a 5'-sequence having the sequence of a tag complementary to the sequence of a tag complement belonging to a family of tag complements of the invention and a $3^{\prime}$-end extending to immediately adjacent the locus;
(b) enzymatically extending the $3^{\prime}$-end of the primer in the presence of $a$ plurality of nucleoside triphosphate derivatives each of which is: (i) capable of enzymatic incorporation onto the $3^{\prime}$-end of a growing nucleotide strand; (ii) causes termination of said extension; and (iii) capable of differential detection, one from the other, wherein there is a said derivative complementary to each possible nucleotide present at said locus;
(c) specifically hybridizing the extended primer formed in step (b) to a tag complement having the tag complement sequence of (a); and
(d) detecting the nucleotide derivative incorporated into the primer in step (b) so as to identify the base located at the locus of the nucleic acid molecule;
wherein each tag of (a) is unique for each nucleic acid molecule and steps (a) and (b) are carried out with said nucleic molecules in the presence of each other. The derivative can be a dideoxy nucleoside triphosphate.

Each respective complement can be attached as a uniform population of substantially identical complements in spacially discrete regions on one or more solid phase support(s).

Each tag complement can include a label, each such label being exposing a sample of the mixture to the detection moiety under conditions suitable to permit (or cause) said specific binding of the molecule and target;
(iv) providing a family of suitable tag complements of the invention wherein the family contains a first tag complement having a sequence complementary to that of the first tag;
(v) exposing the sample to the family of tag complements under conditions suitable to permit (or cause) specific hybridization of the first tag and its tag complement;
(vi) determining whether a said first detection moiety hybridized to a first said tag complement is bound to a said labelled target in order to determine the presence or absence of said target in the mixture.
Preferably, the first tag complement is linked to a solid support at a specific location of the support and step (vi) includes detecting the presence of the first label at said specified location.

Also, the first tag complement can include a second label and step (vi) includes detecting the presence of the first and second labels in a hybridized complex of the moiety and the first tag complement.

Further, the target can be selected from the group consisting of organic molecules, antigens, proteins, polypeptides, antibodies and nucleic acids. The target can be an antigen and the first molecule can be an antibody specific for that antigen.

The antigen is usually a polypeptide or protein and the labelling step can include conjugation of fluorescent molecules, digoxigenin, biotinylation and the like.

The target can be a nucleic acid and the labelling step can

## DETAILED DESCRIPTION OF THE INVENTION

 FIGURESReference is made to the attached figures in which,
Figure 1 illustrates generally the steps followed to obtain a family of sequences of the present invention;

Figure 2 shows the intensity of the signal (MFI) for each perfectly matched sequence (probe sequences indicated in Table I) and its complement (target, at 50 fmol) obtained as described in Example 1 ;

Figure 3 is a three dimensional representation showing crosshybridization observed for the sequences of Figure 2 as described in Example 1. The results shown in Figure 2 are reproduced along the diagonal of the drawing; and

Figure 4 is illustrative of results obtained for an individual target (SEQ ID NO:90, target No. 90) when exposed to the 100 probes of Example 1. The MFI for each bead is plotted.

## DETAILED EMBODIMENTS

The invention provides a method for sorting complex mixtures of molecules by the use of families of oligonucleotide sequence tags. The families of oligonucleotide sequence tags are designed so as to provide minimal cross hybridization during the sorting process. Thus any sequence within a family of sequences will not significantly cross-hybridize with any other sequence derived from that family under appropriate hybridization conditions known by those skilled in the art. The invention is particularly useful in highly parallel processing of analytes.

## Families of Oligonucleotide Sequence Tags

The present invention includes a family of 24 mer polynucleotides that have been demonstrated to be minimally cross-hybridizing with each other. This family of polynucleotides is thus useful as a family of tags, and their complements as tag complements.

In order to be considered for inclusion into the family, a sequence had to satisfy a certain number of rules regarding its composition. For example, repetitive regions that present potential hybridization problems such as four or more of a similar base (e.g., AAAA or TTTT) or pairs of Gs were forbidden. Another rule is that each sequence contains exactly six Gs and no Cs, in order
to have sequences that are more or less isothermal. Also required for a 24 mer Based on these rules, a computer program can derive families of sequences that exhibit minimal or no cross-hybridization behavior. The exact method used by the computer program is not crucial since various computer programs can derive similar families based on these rules. Such a program is for example described in international patent application No. PCT/CA 01/00141 published under WO $01 / 59151$ on August 16,2001 . Other programs can use different methods, such as the ones summarized below.

A first method of generating a maximum number of minimally crosshybridizing polynucleotide sequences starts with any number of non-crosshybridizing sequences, for example just one sequence, and increases the family as follows. A certain number of sequences is generated and compared to the sequences already in the family. The generated sequences that exhibit too much similarity with sequences already in the family are dropped. Among the "candidate sequences" that remain, one sequence is selected and added to the family. The other candidate sequences are then compared to the selected sequence, and the ones that show too much similarity are dropped. A new sequence is selected from the remaining candidate sequences, if any, and added to the family, and so on until there are no candidate sequences left. At this stage, the process can be repeated (generating a certain number of sequences and comparing them to the sequences in the family, etc.) as often as desired. The family obtained at the end of this method contains only minimally crosshybridizing sequences.

A second method of generating a maximum number of minimally crosshybridizing polynucleotide sequences starts with a fixed-size family of polynucleotide sequences. The sequences of this family can be generated randomly or designed by some other method. Many sequences in this family may
not be compatible with each other, because they show too much similarity and are not minimally cross-hybridizing. Therefore, some sequences need to be replaced by new ones, with less similarity. One way to achieve this consists of repeatedly replacing a sequence of the family by the best (that is, lowest similarity) sequence among a certain number of (for example, randomly generated) sequences that are not part of the family. This process can be repeated until the family of sequences shows minimal similarity, hence minimal cross-hybridizing, or until a set number of replacements has occurred. If, at the end of the process, some sequences do not obey the similarity rules that have been set, they can be taken out of the family, thus providing a somewhat smaller family that only contains minimally cross-hybridizing sequences. Some additional rules can be added to this method in order to make it more efficient, such as rules to determine which sequence will be replaced.

Such methods have been used to obtain the 1168 non-cross-hybridizing tags of Table I that are the subject of this patent application.

One embodiment of the invention is a composition comprising molecules for use as tags or tag complements wherein each molecule comprises an oligonucleotide selected from a set of oligonucleotides based on the group of sequences set out in Table IA, wherein each of the numeric identifiers 1 to 3 (see the Table) is a nucleotide base selected to be different from the others of 1 to 3 . According to this embodiment, several different families of specific sets of oligonucleotide sequences are described, depending upon the assignment of bases made to the numeric identifiers 1 to 3.

The sequences contained in Table I have a mathematical relationship to each other, described as follows.

Let $S$ and $T$ be two DNA sequences of lengths $s$ and $t$ respectively. While the term "alignment" of nucleotide sequences is widely used in the field of biotechnology, in the context of this invention the term has a specific meaning illustrated here. An alignment of $S$ and $T$ is a $2 x p$ matrix $A$ (with $p \geq s$ and $p \geq t$ ) such that the first (or second) row of $A$ contains the characters of $S$ (or $T$ respectively) in order, interspersed with $p-s$ (or $p-t$ respectively) spaces. It assumed that no column of the alignment matrix contains two spaces, i.e., that any alignment in which a column contains two spaces is ignored and not considered here. The columns containing the same base in both rows are called matches, while the columns containing different bases are called mismatches. Each column of an alignment containing a space in its first row is called an insertion and each colmun containing a space in its second row is called a deletion while a column of the alignment containing a space in either
row is called an indel. Insertions and deletions within a sequence are represented by the character '-'. A gap is a continuous sequence of spaces in one of the rows (that is neither immediately preceded nor immediately followed by another space in the same row), and the length of a gap is the number of spaces in that gap. An internal gap is one in which its first space is preceded by a base and its last space is followed by a base and an internal indel is an indel belonging to an internal gap. Finally, a block is a continuous sequence of matches (that is neither immediately preceded nor immediately followed by another match), and the length of $a$ block is the number of matches in that block. In order to illustrate these definitions, two sequences $S=$ TGATCGTAGCTACGCCGCG (of length $\mathrm{s}=19$; SEQ ID NO:1169) and $\mathrm{T}=$ CGTACGATTGCAACGT (of length $t=16$; SEQ ID NO:1170) are considered. Exemplary alignment $R_{1}$ of $S$ and $T$ (with $P=23$ ) is:

## Alignment $\mathrm{R}_{1}$ :

| - | - | - | - | T | G | A | T | C | G | T | A | G | C | T | A | C | G | C | C | G | C | G |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| C | G | T | A | C | G | A | T | - | - | T | - | G | C | A | A | C | G | T | - | - | - | - |

Columns 1 to $4,9,10,12$ and 20 to 23 are indels, columns 6, 7, 8, 11, 13, 14, 16, 17 and 18 are matches, and columns 5, 15 and 19 are mismatches. Columns 9 and 10 form a gap of length 2 , while columns 16 to 18 form a block of length 3. Columns 9, 10 and 12 are internal indels.

A score is assigned to the alignment $A$ of two sequences by assigning weights to each of matches, mismatches and gaps as follows:

- the reward for a match $m$,
- the penalty for a mismatch mm,
- the penalty for opening a gap og,
- the penalty for extending a gap eg.

Once these values are set, a score to each column of the alignment is assigned according to the following rules:

1. assign 0 to each column preceding the first match and to each column following the last match.
2. for each of the remaining columns, assign $m$ if it is a match, $-m m$ if it is a mismatch, $-o g-e g$ if it is the first indel
of a gap, -eg if it is an indel but not the first indel of a gap.

The score of the alignment $A$ is the sum of the scores of its columns. An alignment is said to be of maximum score if no other alignment of the same two sequences has a higher score (with the same values of $m$, $m m$, og and eg). A person knowledgeable in the field will recognize this method of scoring an alignment as scoring a local (as opposed to global) alignment with affine gap penalties (that is, gap penalties that can distinguish between the first indel of a gap and the other indels). It will be appreciated that the total number of indels that open a gap is the same as the total number of gaps and that an internal indel is not one of those assigned a 0 in rule (1) above. It will also be noted that foregoing rule (1) assigns a for non-internal mismatches. An internal mismatch is a mismatch that is preceded and followed (not necessarily immediately) by a match.

As an illustration, if the values of $m, m m, o g$ and eg are set to $3,1,2$ and 1 respectively, alignment $R_{1}$ has a score of 19 , determined as shown below:

Scoring of Alignment $R_{1}$

| - | - | - | - | T | G | A | T | C | G | T | A | G | C | T | A | C | G | C | C | G | C | G |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| C | G | T | A | C | G | A | T | - | - | T | - | G | C | A | A | C | G | T | - | - | - | - |



Note that for two given sequences $S$ and $T$, there are numerous alignments. There are often several alignments of maximum score.

Based on these alignments, five sequence similarity measures are defined as follows. For two sequences $S$ and $T$, and weights $\{m, m m, o g$, eg \}:

- M1 is the maximum number of matches over all alignments free of internal indels;
- M2 is the maximum length of a block over all alignments;
- M3 is the maximum number of matches over all alignments of maximum score;
- M4 is the maximum sum of the lengths of the longest two blocks over all alignments of maximum score;
- M5 is the maximum sum of the lengths of all the blocks of length at least 3, over all alignments of maximum score.
Notice that, by definition, the following inequalities between these similarity measures are obtained: M4 $\leq M 3$ and $M 5 \leq M 3$. Also, in order to determine $M 2$ it is sufficient to determine the maximum length of a block over all alignments free of internal indels. For two given sequences, the values of $M 3$ to $M 5$ can vary depending on the values of the weights $\{m, m m, o g, e g\}$, but not $M 1$ and $M 2$.

For weights $\{3,1,2,1\}$, the illustrated alignment is not a maximum score alignment of the two example sequences. But for weights $\{6,6,0,6\}$ it is; hence this alignment shows that for these two example sequences, and weights $\{6,6,0,6\}, M 2 \geq 3, M 3 \geq 9, M 4 \geq 6$ and $M 5 \geq 6$. In order to determine the exact values of M1 to M5, all the necessary alignments need to be considered. $M 1$ and $M 2$ can be found by looking at the $s+t-1$ alignments free of internal indels, where $s$ and $t$ are the lengths of the two sequences considered. Mathematical tools known as dynamic programming can be implemented on a computer and used to determine M3 to M5 in a very quick way. Using a computer program to do these calculations, it was determined that:

- with the weights $\{6,6,0,6\}, M 1=8, M 2=4, M 3=10, M 4=6$ and M5 = 6;
- with the weights $\{3,1,2,1\}, M 1=8, M 2=4, M 3=10, M 4=6$ and $M 5=4$.

According to the preferred embodiment of this invention, two sequences $S$ and $T$ each of length 24 are too similar if at least one of the following happens:

- MI > 16 or
- M2 > 13 or
- M3 > 20 or
- M4 > 16 or
- $M 5>19$
when using either weights $\{6,6,0,6\}$, or $\{6,6,5,1\}$, or $\{6,2,5$, $1\}$, or $\{6,6,6,0\}$. In other words, the five similarity measures between $S$ and $T$ are determined for each of the above four sets of weights, and checked against these thresholds (for a total of 20 tests).

The above thresholds of $16,13,20,16$ and 19 , and the above sets of weights, were used to obtain the sequences listed in Table I.
Additional sequences can thus be added to those of Table I as long as the above alignment rules are obeyed for all sequences.

It is also possible to alter thresholds M1, M2, etc., while remaining within the scope of this invention. It is thus possible to substitute or add sequences to those of Table $I$, or more generally to those of Table IA to obtain other sets of sequences that would also exhibit reasonably low cross-hybridization. More specifically, a set of 24 mer sequences in which there are no two sequences that are too similar, where too similar is defined as:

- M1 > 19 or
- $M 2>17$ or
- M3 > 21 or
- $M 4>18$ or
- M5 > 20
when using either weights $\{6,6,0,6\}$, or $\{6,6,5,1\}$, or $\{6,2,5$, $1\}$, or $\{6,6,6,0\}$, would also exhibit low cross-hybridization. Reducing any of the threshold values provides sets of sequences with even lower cross-hybridization. Alternatively, 'too similar' can also be defined as:
- M1 > 19 or
- M2 > 17 or
- M3 > 21 or
- M4 > 18 or
- M5 > 20
when using either weights $\{3,1,2,1\}$. Alternatively, other combinations of weights will lead to sets of sequences with low crosshybridization.

Notice that using weights $\{6,6,0,6\}$ is equivalent to using weights $\{1,1,0,1\}$, or weights $\{2,2,0,2\}, \ldots$ (that is, for any two sequences, the values of M1 to M5 are exactly the same whether weights $\{6,6,0,6\}$ or $\{1,1,0,1\}$ or $\{2,2,0,2\}$ or any other multiple of $\{1,1,0,1\}$ is used).

When dealing with sequences of length other than 24 , or sequences of various lengths, the definition of similarity can be adjusted. Such adjustments are obvious to the persons skilled in the art. For example, when comparing a sequence of length L 1 with a sequence of length L 2 (with Ll<L2), they can be considered as too similar when

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Ml > 19/24 x Ll
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M2 $>17 / 24 \mathrm{x} \mathrm{L1}$
M3 $>21 / 24 \times \mathrm{L} 1$
M4 > 18/24 x L1

M5 > 20/24 x LI
when using either weights $\{6,6,0,6\}$, or $\{6,6,5,1\}$, or $\{6,2,5,1\}$ or $\{6,6,6,0\}$.

Polynucleotide sequences can be composed of a subset of natural bases most preferably A, T and G. Sequences that are deficient in one base possess useful characteristics, for example, in reducing potential secondary structure formation or reduced potential for cross hybridization with nucleic acids in nature. Also, it is preferable to have tag sequences that behave isothermally. This can be achieved for example by maintaining a constant base composition for all sequences such as six $G s$ and eighteen As or $T s$ for each sequence. Additional sets of sequences can be designed by extrapolating on the original family of non-cross-hybridizing sequences by simple methods known to those skilled in the art.

In order to validate the sequence set, a subset of sequences from the family of 1168 sequence tags was selected and characterized, in terms of the ability of these sequences to form specific duplex structures with their complementary sequences, and the potential for cross-hybridization within the sequence set. See Example 1, below. The subset of 100 sequences was randomly selected, and analyzed using the Luminex ${ }^{100}$ LabMAP ${ }^{T M}$ platform. The 100 sequences were chemically immobilized onto the set of 100 different Luminex microsphere populations, such that each specific sequence was coupled to one spectrally distinct microsphere population. The pool of 100 microsphere-immobilized probes was then hybridized with each of the 100 corresponding complementary sequences. Each sequence was examined individually for its specific hybridization with its complementary sequence, as well as for its non-specific hybridization with the other 99 sequences present in the reaction. This analysis demonstrated the propensity of each sequence to hybridize only to its complement (perfect match), and not to cross-hybridize appreciably with any of the other oligonucleotides present in the hybridization reaction.

It is within the capability of a person skilled in the art, given the family of sequences of Table $I$, to modify the sequences, or add other sequences while largely retaining the property of minimal cross-hybridization which the polynucleotides of Table I have been demonstrated to have.

There are 1168 polynucleotide sequences given in Table I. Since all
1168 of this family of polynucleotides can work with each other as a minimally cross-hybridizing set, then any plurality of polynucleotides that is a subset of the 1168 can also act as a minimally cross-hybridizing set of polynucleotides. An application in which, for example, 30 molecules are to be
sorted using a family of polynucleotide tags and tag complements could thus use any group of 30 sequences shown in Table I. This is not to say that some subsets may be found in a practical sense to be more preferred than others. For example, it may be found that a particular subset is more tolerant of a wider variety of conditions under which hybridization is conducted before the degree of cross-hybridization becomes unacceptable.

It may be desirable to use polynucleotides that are shorter in length than the 24 bases of those in Table I. A family of subsequences (i.e., subframes of the sequences illustrated) based on those contained in Table I having as few as 10 bases per sequence could be chosen, so long as the subsequences are chosen to retain homological properties between any two of the sequences of the family important to their non cross-hybridization

The selection of sequences using this approach would be amenable to a computerized process. Thus for example, a string of 10 contiguous bases of
 NO:1)

The same string of contiguous bases from the second 24 mer could then be selected and compared for similarity against the first chosen sequence: GTTAGAGTTAATTGTATTTGATGA (SEQ ID NO:2). A systematic pairwise comparison could then be carried out to determine if the similarity requirements are violated. If the pair of sequences does not violate any set property, a lomer subsequence can be selected from the third 24 mer sequence of Table $I$, and compared to each of the first two lomer sequences (in a pairwise fashion to determine its compatibility therewith, etc. In this way a family of 10 mer sequences may be developed.

It is within the scope of this invention, to obtain families of sequences containing limer, $12 \mathrm{mer}, 13 \mathrm{mer}, 14 \mathrm{mer}, 15 \mathrm{mer}, 16 \mathrm{mer}, 17 \mathrm{mer}, 18 \mathrm{mer}$, 19 mer , 2 mer, 21 mer, 22 mer and 23 mer sequences by analogy to that shown for 10 mer sequences.

It may be desirable to have a family of sequences in which there are sequences greater in length than the 24 mer sequences shown in Table I. It is within the capability of a person skilled in the art, given the family of sequences shown in Table $I$, to obtain such a family of sequences. One possible approach would be to insert into each sequence at one or more locations a nucleotide, non-natural base or analogue such that the longer sequence should not have greater similarity than any two of the original non-cross-hybridizing sequences of Table I and the addition of extra bases to the tag sequences should not result in a major change in the thermodynamic properties of the tag sequences of that set for example the GC content must be maintained between $10 \%-40 \%$ with a variance from the average of $20 \%$. This
method of inserting bases could be used to obtain, for example, a family of sequences up to 40 bases long.

Given a particular family of sequences that can be used as a family of tags (or tag complements), e.g., those of Table I, a skilled person will readily recognize variant families that work equally as well.

Again taking the sequences of Table $I$ for example, every $T$ could be converted to an $A$ and vice versa and no significant change in the crosshybridization properties would be expected to be observed. This would also be true if every $G$ were converted to a $C$.

Also, all of the sequences of a family could be taken to be constructed in the 5'-3' direction, as is the convention, or all of the constructions of sequences could be in the opposition direction ( $3^{\prime}-5^{\prime}$ ).

There are additional modifications that can be carried out. For example, $C$ has not been used in the family of sequences. Substitution of $C$ in place of one or more $G^{\prime}$ s of a particular sequence would yield a sequence that is at least as low in homology with every other sequence of the family as was the particular sequence chosen for modification. It is thus possible to substitute $C$ in place of one or more $G$ 's in any of the sequences shown in Table I. Analogously, substituting of $C$ in place of one or more $A^{\prime} s$ is possible, or substituting $C$ in place of one or $T$ 's is possible.

It is preferred that the sequences of a given family are of the same, or roughly the same length. Preferably, all the sequences of a family of sequences of this invention have a length that is within five bases of the base-length of the average of the family. More preferably, all sequences are within four bases of the average base-length. Even more preferably, all or almost all sequences are within three bases of the average base-length of the family. Better still, all or almost all sequences have a length that is within two of the base-length of the average of the family, and even better still, within one of the base-length of the average of the family.

It is also possible for a person skilled in the art to derive sets of sequences from the family of sequences described in this specification and remove sequences that would be expected to have undesirable hybridization properties.

## Methods For Synthesis Of Oligonucleotide Families

Preferably oligonucleotide sequences of the invention are synthesized directly by standard phosphoramidite synthesis approaches and the like (Caruthers et al, Methods in Enzymology; 154, 287-313: 1937; Lipshutz et al, Nature Genet.; 21, 20-24: 1999; Fodor et al, Science; 251, 763-773: 1991). Alternative chemistries involving non
natural bases such as peptide nucleic acids or modified nucleosides that offer advantages in duplex stability may also be used (Hacia et al; Nucleic Acids Res ;27: 4034-4039, 1999; Nguyen et al, Nucleic Acids Res.;27, 1492-1498: 1999; Weiler et al, Nucleic Acids Res.; 25, 2792- 2799:1997). It is also possible to synthesize the oligonucleotide sequences of this invention with alternate nucleotide backbones such as phosphorothioate or phosphoroamidate nucleotides. Methods involving synthesis through the addition of blocks of sequence in a stepwise manner may also be employed (Lyttle et al, Biotechniques, 19: 274-280 (1995). Synthesis may be carried out directly on the substrate to be used as a solid phase support for the application or the oligonucleotide can be cleaved from the support for use in solution or coupling to a second support.

## Solid Phase Supports

There are several different solid phase supports that can be used with the invention. They include but are not limited to slides, plates, chips, membranes, beads, microparticles and the like. The solid phase supports can also vary in the materials that they are composed of including plastic, glass, silicon, nylon, polystyrene, silica gel, latex and the like. The surface of the support is coated with the complementary tag sequences by any conventional means of attachment.

In preferred embodiments, the family of tag complement sequences is derivatized to allow binding to a solid support. Many methods of derivatizing a nucleic acid for binding to a solid support are known in the art (Hermanson G., Bioconjugate Techniques; Acad. Press: 1996). The sequence tag may be bound to a solid support through covalent or non-covalent bonds (Iannone et al, Cytometry; 39: 131-140, 2000; Matson et al, Anal. Biochem.; 224: 110-106, 1995; Proudnikov et al, Anal Biochem; 259: 34-41, 1998; Zammatteo et al, Analytical Biochemistry; 280:143-150, 2000). The sequence tag can be conveniently derivatized for binding to a solid support by incorporating modified nucleic acids in the terminal $5^{\prime}$ or $3^{\prime}$ locations.

A variety of moieties useful for binding to a solid support (e.g., biotin, antibodies, and the like), and methods for attaching them to nucleic acids, are known in the art. For example, an amine-modified nucleic acid base (available from, eg., Glen Research) may be attached to a solid support (for example, Covalink-NH, a polystyrene surface grafted with secondary amino groups, available from Nunc) through a bifunctional crosslinker (e.g., bis(sulfosuccinimidyl suberate), available from Pierce). Additional spacing
moieties can be added to reduce steric hindrance between the capture moiety and the surface of the solid support.

## Attaching Tags to Analytes for Sorting

A family of oligonucleotide tag sequences can be conjugated to a population of analytes most preferably polynucleotide sequences in several different ways including but not limited to direct chemical synthesis, chemical coupling, ligation, amplification, and the like. Sequence tags that have been synthesized with primer sequences can be used for enzymatic extension of the primer on the target for example in $P C R$ amplification.

## Detection of Single Nucleotide Polymorphisms Using Primer Extension

There are a number of areas of genetic analysis where families of non-cross-hybridizing sequences can be applied including disease diagnosis, single nucleotide polymorphism analysis, genotyping, expression analysis and the like. One such approach for genetic analysis, referred to as the primer extension method (also known as Genetic Bit Analysis (Nikiforov et al, Nucleic Acids Res.; 22, 4167-4175: 1994; Head et al Nucleic Acids Res.; 25, 5065-5071: 1997)), is an extremely accurate method for identification of the nucleotide located at a specific polymorphic site within genomic DNA. In standard primer extension reactions, a portion of genomic DNA containing a defined polymorphic site is amplified by PCR using primers that flank the polymorphic site. In order to identify which nucleotide is present at the polymorphic site, a third primer is synthesized such that the polymorphic position is located
immediately $3^{\prime}$ to the primer. A primer extension reaction is set up containing the amplified DNA, the primer for extension, up to 4 dideoxynucleoside triphosphates (each labeled with a different fluorescent dye) and a DNA polymerase such as the Klenow subunit of DNA Polymerase 1. The use of dideoxy nucleotides ensures that a single base is added to the $3^{\prime}$ end of the primer, a site corresponding to the polymorphic site. In this way the identity of the nucleotide present at a specific polymorphic site can be determined by the identity of the fluorescent dye-labeled nucleotide that is incorporated in each reaction. One major drawback to this approach is its low throughput. Each primer extension reaction is carried out independently in a separate tube.

Universal sequences can be used to enhance the throughput of primer extension assay as follows. A region of genomic DNA containing multiple polymorphic sites is amplified by PCR. Alternatively, several genomic regions containing one or more polymorphic sites each are amplified together in a multiplexed $P C R$ reaction. The primer extension reaction
is carried out as described above except that the primers used are chimeric, each containing a unique universal tag at the $5^{\prime}$ end and the sequence for extension at the $3^{\prime}$ end. In this way, each gene-specific sequence would be associated with a specific universal sequence. The chimeric primers would be hybridized to the amplified DNA and primer extension is carried out as described above. This would result in a mixed pool of extended primers, each with a specific fluorescent dye characteristic of the incorporated nucleotide. Following the primer extension reaction, the mixed extension reactions are hybridized to an array containing probes that are reverse complements of the universal sequences on the primers. This would segregate the products of a number of primer extension reactions into discrete spots. The fluorescent dye present at each spot would then identify the nucleotide incorporated at each specific location. A number of additional methods for the detection of single nucleotide polymorphisms, including but not limited to, allele specific polymerase chain reaction (ASPCR), allele specific primer extension (ASPE) and oligonucleotide ligation assay (OLA) can be performed by someone skilled in the art in combination with the tag sequences described herein.

## Rits Using Families Of Tag Sequences

The families of non cross-hybridizing sequences may be provided in kits for use in for example genetic analysis. Such kits include at least one set of non-cross-hybridizing sequences in solution or on a solid support. Preferably the sequences are attached to microparticles and are provided with buffers and reagents that are appropriate for the application. Reagents may include enzymes, nucleotides, fluorescent labels and the like that would be required for specific applications. Instructions for correct use of the kit for a given application will be provided.

## EXAMPLES

EXAMPLE 1 - Cross Talk Behavior of Sequence on Beads
A group of 100 sequences, randomly selected from Table $I$, was tested for feasibility for use as a family of minimally cross-hybridizing oligonucleotides. The 100 sequences selected are separately indicated in Table I along with the numbers assigned to the sequences in the tests.

The tests were conducted using the Luminex LabMAP ${ }^{T M}$ platform available from Luminex Corporation, Austin, Texas, U.S.A. The one hundred sequences, used as probes, were synthesized as oligonucleotides by Integrated DNA Technologies (IDT, Coralville, Iowa, U.S.A.). Each probe included a $C_{6}$
aminolink group coupled to the $5^{\prime}$-end of the oligonucleotide through a $C_{12}$ ethylene glycol spacer. The $C_{6}$ aminolink molecule is a six carbon spacer containing an amine group that can be used for attaching the oligonucleotide to a solid support. One hundred oligonucleotide targets (probe complements), the sequence of each being the reverse complement of the 100 probe sequences, were also synthesized by IDT. Each target was labelled at its 5'-end with biotin. All oligonucleotides were purified using standard desalting procedures, and were reconstituted to a concentration of approximately $200 \mu \mathrm{M}$ in sterile, distilled water for use. Oligonucleotide concentrations were determined spectrophotometrically using extinction coefficients provided by the supplier.

Each probe was coupled by its amino linking group to a carboxylated fluorescent microsphere of the LapMAP system according to the Luminex ${ }^{200}$ protocol. The microsphere, or bead, for each probe sequence has unique, or spectrally distinct, light absorption characteristics which permits each probe to be distinguished from the other probes. Stock bead pellets were dispersed by sonication and then vortexing. For each bead population, five million microspheres ( $400 \mu \mathrm{~L}$ ) were removed from the stock tube using barrier tips and added to a 1.5 mL Eppendorf tube (USA Scientific). The microspheres were then centrifuged, the supernatant was removed, and beads were resuspended in $25 \mu \mathrm{~L}$ of $0.2 \mathrm{M} \operatorname{MES}$ (2-(N-morpholino)ethane sulfonic acid) (Sigma), pH 4.5, followed by vortexing and sonication. One nmol of each probe (in a $25 \mu \mathrm{~L}$ volume) was added to its corresponding bead population. A volume of $2.5 \mu \mathrm{~L}$ of EDC cross-linker (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Pierce), prepared immediately before use by adding 1.0 mL of sterile $\mathrm{ddH}_{2} \mathrm{O}$ to 10 mg of EDC powder, was added to each microsphere population. Bead mixes were then incubated for 30 minutes at room temperature in the dark with periodic vortexing. A second 2.5 $\mu \mathrm{L}$ aliquot of freshly prepared EDC solution was then added followed by an additional 30 minute incubation in the dark. Following the second EDC incubation, 1.0 mL of $0.02 \%$ Tween -20 (BioShop) was added to each bead mix and vortexed. The microspheres were centrifuged, the supernatant was removed, and the beads were resuspended in 1.0 mL of $0.1 \%$ sodium dodecyl sulfate (Sigma). The beads were centrifuged again and the supernatant removed. The coupled beads were resuspended in 100 $\mu \mathrm{L}$ of 0.1 M MES pH 4.5 . Bead concentrations were then determined by diluting each preparation 100 -fold in $d_{2} \mathrm{H}_{2} \mathrm{O}$ and enumerating using a

Neubauer BrightLine Hemacytometer. Coupled beads were stored as individual populations at $80^{\circ} \mathrm{C}$ protected from light.

The relative oligonucleotide probe density on each bead population was assessed by Terminal Deoxynucleotidyl Transferase (TdT) endlabelling with biotin-ddUTPs. TdT was used to label the $3^{\prime}$-ends of single-stranded DNA with a labeled ddNTP. Briefly, $180 \mu \mathrm{~L}$ of the pool of 100 bead populations (equivalent to about 4000 of each bead type) to be used for hybridizations was pipetted into an Eppendorf tube and centrifuged. The supernatant was removed, and the beads were washed in $1 x$ TdT buffer. The beads were then incubated with a labelling reaction mixture, which consisted of $5 x$ TdT buffer, $25 \mathrm{mM} \mathrm{CoCl} \mathrm{C}_{2}$, and 1000 pmol of biotin-16-ddUTP (all reagents were purchased from Roche). The total reaction volume was brought up to $85.5 \mu \mathrm{~L}$ with sterile, distilled $\mathrm{H}_{2} \mathrm{O}$, and the samples were incubated in the dark for 1 hour at $37^{\circ} \mathrm{C}$. A second aliquot of enzyme was added, followed by a second 1 hour incubation. Samples were run in duplicate, as was the negative control, which contained all components except the TdT. In order to remove unincorporated biotin-ddUTP, the beads were washed 3 times with $200 \mu \mathrm{~L}$ of hybridization buffer, and the beads were resuspended in $50 \mu \mathrm{~L}$ of hybridization buffer following the final wash. The biotin label was detected spectrophotometrically using SA-PE (streptavidin-phycoerythrin conjugate). The streptavidin binds to biotin and the phycoerythrin is spectrally distinct from the probe beads. The $10 \mathrm{mg} / \mathrm{mL}$ stock of SA -PE was diluted 100 -fold in hybridization buffer, and $15 \mu \mathrm{~L}$ of the diluted SA-PE was added directly to each reaction and incubated for 15 minutes at $37^{\circ} \mathrm{Celsius} .\mathrm{The} \mathrm{reactions} \mathrm{were} \mathrm{analyzed} \mathrm{on} \mathrm{the} \mathrm{Luminex}{ }^{100}$ LabMAP. Acquisition parameters were set to measure 100 events per bead using a sample volume of $50 \mu \mathrm{~L}$.

The results obtained are shown in Figure 2. As can be seen the Mean Fluorescent Intensity (MFI) of the beads varies from 840.3 to 3834.9, a range of 4.56-fold. Assuming that the labelling reactions are complete for all of the oligonucleotides, this illustrates the signal intensity that would be obtained for each type of bead at this concentration if the target (i.e., labelled complement) was bound to the probe sequence to the full extent possible.

The cross-hybridization of targets to probes was evaluated as follows. 100 oligonucleotide probes linked to 100 different bead populations, as described above, were combined to generate a master bead mix, enabling multiplexed reactions to be carried out. The pool of
microsphere-immobilized probes was then hybridized individually with each biotinylated target. Thus, each target was examined individually for its specific hybridization with its complementary bead-immobilized sequence, as well as for its non-specific hybridization with the other each hybridization reaction, $25 \mu \mathrm{~L}$ bead mix (containing about 2500 of each bead population in hybridization buffer) was added to each well of a 96-well Thermowell PCR plate and equilibrated at $37^{\circ} \mathrm{C}$. Each target was diluted to a final concentration of $0.002 \mathrm{fmol} / \mu \mathrm{L}$ in hybridization buffer, and $25 \mu \mathrm{~L}$ ( 50 fmol ) was added to each well, giving a final reaction volume of $50 \mu \mathrm{~L}$. Hybridization buffer consisted of 0.2 M NaCl , 0.1 M Tris, $0.08 \%$ Triton $\mathrm{X}-100, \mathrm{pH} 8.0$ and hybridizations were performed at $37^{\circ} \mathrm{C}$ for 30 minutes. Each target was analyzed in triplicate and six background samples (i.e. no target) were included in each plate. A SA$P E$ conjugate was used as a reporter, as described above. The $10 \mathrm{mg} / \mathrm{mL}$ stock of SA-PE was diluted 100 -fold in hybridization buffer, and $15 \mu \mathrm{~L}$ of the diluted SA-PE was added directly to each reaction, without removal of unbound target, and incubated for 15 minutes at $37^{\circ} \mathrm{C}$. Finally, an additional $35 \mu \mathrm{~L}$ of hybridization buffer was added to each well, resulting in a final volume of $100 \mu \mathrm{~L}$ per well prior to analysis on the Luminex ${ }^{100}$ LabMAP. Acquisition parameters were set to measure 100 events per bead using a sample volume of $80 \mu \mathrm{~L}$.

The percent hybridization was calculated for any event in which the NET MFI was at least 3 times the zero target background. In other words, a calculation was made for any sample where (MFI $_{\text {sample }}-\mathrm{MFI}_{\text {zero target }}$ background $) / \mathrm{MFI}_{\text {zero target background }} \geq 3$.

The net median fluorescent intensity ( $\mathrm{MFI}_{\text {sample }}-\mathrm{MFI}_{\text {zero target background }}$ ) generated for all of the 10,000 possible target/probe combinations was calculated. As there are 100 probes and 100 targets, there are $100 \times 100$ $=10,0000$ possible different interactions possible of which 100 are the result of perfect hybridizations. The remaining 9900 result from hybridization of a target with a mismatched probe. A cross-
hybridization event is then defined as a non-specific event whose net median fluorescent intensity exceeds 3 times the zero target background. In other words, a cross-talk calculation is only be made for any sample where ( $M F I_{\text {sample }}-M F I_{\text {zero target background }}$ )/MFI zero target background $\geq 3$. Cross hybridization events were quantified by expressing the value of the cross-hybridization signal as a percentage of the perfect match hybridization signal with the same probe.

The results obtained are illustrated in Figure 3. The ability of each target to be specifically recognized by its matching probe is shown. Of the possible 9900 non-specific hybridization events that could have occurred when the 100 targets were each exposed to the pool of 100 probes, 6 events were observed. Of these 6 events, the highest non-specific event generated a signal equivalent to $5.3 \%$ of the signal observed for the perfectly matched pair (i.e. specific hybridization event).

Each of the 100 targets was thus examined individually for specific hybridization with its complement sequence as incorporated onto a microsphere, as well as for non-specific hybridization with the complements of the other 99 target sequences. Representative hybridization results for target (complement of probe 90, Table I) are shown in Figure 4. Probe 90 was found to hybridize only to its perfectly-matched target. No cross-hybridization with any of the other 99 targets was observed.

The foregoing results demonstrate the possibility of incorporating the 1168 sequences of Table $I$, or any subset thereof, into a multiplexed system with the expectation that most if not all sequences can be distinguished from the others by hybridization. That is, it is possible to distinguish each target from the other targets by hybridization of the target with its precise complement and minimal hybridization with complements of the other targets.

EXAMPLE 2 - Tag sequences used in sorting polynucleotides
The family of non cross hybridizing sequence tags or a subset thereof can be attached to oligonucleotide probe sequences during synthesis and used to generate amplified probe sequences. In order to test the feasibility of PCR amplification with non cross hybridizing sequence tags and subsequently addressing each respective sequence to its appropriate location on twodimensional or bead arrays, the following experiment was devised. A 24 mer tag sequence can be connected in a $5^{\prime}-3^{\prime}$ specific manner to a p53 exon specific sequence ( 20 mer reverse primer). The connecting p53 sequence represents the inverse complement of the nucleotide gene sequence. To facilitate the subsequent generation of single stranded DNA post-amplification the tagReverse primer can be synthesized with a phosphate modification ( $\mathrm{PO}_{4}$ ) on the 5'-end. A second PCR primer can also be generated for each desired exon, represented by the Forward ( $5^{\prime}-3^{\prime}$ ) amplification primer. In this instance the Forward primer can be labeled with a 5'-biotin modification to allow detection with Cy3-avidin or equivalent.

A practical example of the aforementioned description is as follows: For exon 1 of the human p53 tumor suppressor gene sequence the following tagReverse primer (SEQ ID NO:1171) can be generated:

## 222087

222063
5'-PO4-ATGTTAAAGTAAGTGTTGAAATGT -TCCAGGGAAGCGTGTCACCGTCGT-3'
Tag Sequence \# 3
Exon 1 Reverse

The numbering above the Exon-1 reverse primer represents the genomic nucleotide positions of the indicated bases. The corresponding Exon-1 Forward primer sequence (SEQ ID NO:1172) is as follows:

5'-Biotin-TCATGGCGACTGTCCAGCTTTGTG-3'

In combination these primers will amplify a product of 214 bp plus a 24 bp tag extension yielding a total size of 238 bp.
Once amplified, the PCR product can be purified using a QIAquick PCR purification kit and the resulting DNA can be quantified. To generate single stranded DNA, the DNA is subjected to $\lambda$-exonuclease digestion thereby resulting in the exposure of a single stranded sequence (antitag) complementary to the tag-sequence covalently attached to the solid phase array. The resulting product is heated to $95^{\circ} \mathrm{C}$ for 5 minutes and then directly applied to the array at a concentration of $10-50 \mathrm{nM}$. Following hybridization and concurrent sorting, the tag-Exon 1 sequences are visualized using Cy3-streptavidin. In addition to direct visualization of the biotinylated product, the product itself can now act as a substrate for further analysis of the amplified region, such as SNP detection and haplotype determination.

## DEFINITIONS

Non-cross-hybridization: Describes the absence of hybridization between two sequences that are not perfect complements of each other.

Cross-hybridization: The hydrogen bonding of a single-stranded DNA sequence that is partially but not entirely complementary to a singlestranded substrate.

Homology or Similarity: How closely related two or more separate strands of DNA are to each other, based on their base sequences.

Analogue: The symbols $A, G, T / U, C$ take on their usual meaning in the art here. In the case of $T$ and $U$, a person skilled in the art would understand that these are equivalent to each other with respect to the inter-strand hydrogen-bond (watson-Crick) binding properties at work in the context of this invention. The two bases are thus interchangeable and hence the designation of $T / U$. A chemical, which resembles a nucleotide base is an analogue thereof. A base that does not normally appear in DNA but can substitute for the ones, which do, despite minor differences in structure. Analogues particularly useful in this invention are of the naturally occurring bases can be inserted in their respective places where desired. Such an analogue is any non-natural base, such as peptide nucleic acids and the like that undergoes normal Watson-Crick pairing in the same way as the naturally occurring nucleotide base to which it corresponds.

Complement: The opposite or "mirror" image of a DNA sequence. A complementary DNA sequence has an "A" for every "T" and a "C" for every "G". Two complementary strands of single stranded DNA, for example a tag sequence and its complement, will join to form a double-stranded molecule.

Complementary DNA (cDNA): DNA that is synthesized from a messenger RNA template; the single- stranded form is often used as a probe in physical mapping.

Oligonucleotide: Refers to a short nucleotide polymer whereby the nucleotides may be natural nucleotide bases or analogues thereof.

Tag: Refers to an oligonucleotide that can be used for specifically sorting analytes with at least one other oligonucleotide that when used together do not cross hybridize.

Table I
Sequence SEQ ID NO: NO. in
AAATTGTGAAAGATTGTTTGTGTA1 1
GTTAGAGTTAATTGTATTTGATGA1 2
 $T G A T G T T A G A A G T A T A T T G T G A A T \quad 4 \quad-$ TTTGTGTAGAATATGTGTTGTTAAM
AAGAGTATTTGTTGTGAGTTAAAT
GTGTTTATGTTATATGTGAAGTTT
AAAGAGAATAGAATATGTGTAAGT
TATGAAAGAGTGAGATAATGTTTA
ATGAGAAATATGTTAGAATGTGAT
TTAGTTGTTGATGTTTAGTAGTTT
GTAAAGAGTATAAGTTTGATGATA
AAAGTAAGAATGATGTAATAAGTG
GTAGAAATAGTTTATTGATGATTG
TGTAAGTGAAATAGTGAGTTATTT
AAATAGATGATATAAGTGAGAATG
ATAAGTTATAAGTGTTATGTGAGT
TATAGATAAAGAGATGATTTGTTG
AGAGTTGAGAATGTATAGTATTAT
AAGTAGTTTGTAAGAATGATTGTA
TTATGAAATTGAGTGAAGATTGAT
GTATATGTAAATTGTTATGTTGAG
GAATTGTATAAAGTATTAGATGTG
TAGATGAGATTAAGTGTTATTTGA
GTTAAGTTTGTTTATGTATAGAAG
GAGTATTAGTAAAGTGATATGATA
GTGAATGATTTAGTAAATGATTGA
GATTGAAGTTATAGAAATGATTAG
AGTGATAAATGTTAGTTGAATTGT
TATATAGTAAATGTTTGTGTGTTG
TTAAGTGTTAGTTATTTGTTGTAG
GTAGTAATATGAAGTGAGAATATA
$T A G T G T A T A G A A T G T A G A T T T A G T$
TTGTAGATTAGATGTGTTTGTAAA
-
-
TAGTATAGAGTAGAGATGATATTT
ATTGTGAAAGAAAGAGAAGAAATT
TGTGAGAATTAAGATTAAGAATGT
ATATTAGTTAAGAAAGAAGAGTTG
$T \mathrm{~T} G \mathrm{TAGTTGAGAAATATGTAGTTT}$
TAGAGTTGTTAAAGAGTGTAAATA
GTTATGATGTGTATAAGTAATATG
TTTGTTAGAATGAGAAGATTTATG
10
AGTATAGTTTAAAGAAGTAGTAGA
GTGAGATATAGATTTAGAAAGTAA
TTGTTTATAGTGAAGTGAATAGTA
AAGTAAGTAGTAATAGTGTGTTAA
ATTTGTGAGTTATGAAAGATAAGA
GAAAGTAGAGAATAAAGATAAGAA
ATTTAAGATTGTTAAGAGTAGAAG
GTTTAAAGATTGTAAGAATGTGTA
TTTGTGAAGATGAAGTATTTGTAT
TGTGTTTAGAATTTAGTATGTGTA
GATAATGATTATAGAAAGTGTTTG
GTTATTTGTAAGTTAAGATAGTAG
AGTTTATTGAAAGAGTTTGAATAG
TTGTGTTTATTGTGTAGTTTAAAG
ATTGTGAGAAGATATGAAAGTTAT
TGAGAATGTAAAGAATGTTTATTG
ATGTGAAAGTTATGATGTTAATTG
GTTTAGTATTAGTTGTTAAGATTG
GATTGATATTTGAATGTTTGTTTG

Table I
Sequence
SEQ ID NO: NO. in
Ex 1



Table I


Table I

|  | Sequence | SEQ ID NO: | $\begin{gathered} \text { No. in } \\ \text { Ex } 1 \end{gathered}$ |
| :---: | :---: | :---: | :---: |
|  | GTAGTGATATGATTTGAATATTG | G 234 | - |
|  | GTATTGAATTAGAATAGTGAGAA | A 235 | - |
|  | GATATGAGATAGAAGTTTAATGT | T 236 | - |
|  | AAGAAGTAAGTATAAAGTAAATG | G 237 | - |
|  | TtAAGTGTGATAAGAAAGATAGA | A 238 | - |
|  | ATTGTTGAATGTGTTTAAAGAGA | A 239 | 38 |
|  | A ATAATGATGAGATGATTATTGA | A 240 | - |
| T | A GAGAAAGAGAGAATTGTATTAA | A 241 | 39 |
| A | A T TATAATGAGATATGTTTGTGA | A 242 | - |
|  | A TAGATAAGATTGATTGTGTTTG | G 243 | 40 |
| T | Tt TGATGATAATAGAAGAGAATGA | A 244 | - |
| A | A G T GAATAAGTTGTGAATGTTTA | A 245 | - |
| A | A GATGAAAGAAAGTGTAGAATATT | T 246 | - |
| T | -GTTAAATGTATGTAGTAATTGAG | G 247 | 41 |
| T | FAGTAGTGTGAAGTTATTTGTTAT | T 248 | - |
| A | A GTGAATGTTTGTAAAGAGTTTAA | A 249 |  |
| G | GATAAATGAGAATTGAGTAATTG | T 250 | - |
| T | GATGAGAAATTGTTTAAGTGTT | T 251 | - |
| A | A A A TAAGTAGTGTGAGTAATAGTA | A 252 | - |
| T | TATGAAATATGTGATAGTAAGAG | A 253 | - |
| A | TTGTAAGAGTGATTATAGATGA | T 254 | - |
| A | A GAGTAAGAATGAAAGAGATAATA | A 255 | - |
| T | TAAGTAAGTAGATGTTAAAGAGA | T 256 | - |
| A | A A A TAGAAAGAATTGTAGAGTAG | T 257 | - |
| A | A TAGATTTAAGTGAAGAGAGTTA | T 258 | 42 |
| G | GAATGTTTGTAAATGTATAGATA | G 259 | 43 |
| A | A A A TAGAATGAGTAGTGAAATATG | G 260 | - |
| T | TTGAATTATGTAGAGAAAGTAAA | G 261 | - |
| T | A GTAAAT T GAGAGTAGTTGAAT | T 262 | - |
| T | TGTAAAGTGTTTATAGTGTGTAA | T 263 | - |
|  | ATATGATTTGAGATGAGAATGTAA | A 264 | - |
|  | ATATTGATATGTGTTGTGAAGT | A 265 |  |
|  | AGTGAGATTATGAGTATTGATTT | A 266 | 44 |
|  | TGTATTTAGATAGTGAGATTAT | G 267 |  |
|  | A TAGAAATGAAAGATAGATAGAA | G 268 | - |
|  | GATTGTATATGTAAAGTAGTTTA | G 269 |  |
|  | ATGAATGTTATTGTGTGTTGAT | T 270 | 45 |
|  | GATATTAGTAGAGTAAGTATATT | G 271 | - |
|  | GAGATGAATTTGTGTTATGATA | T 272 | - |
|  | ATGAATGAAGTAAAGAGATGTA | A 273 | - |
|  | GAGTGAATTTGTTGTAATTTGTT | T 274 | - |
|  | AGAAATTGTAGAGTTAATTGTGT | A 275 | - |
|  | GTGTTAATGAAAGTTGTGAATAA | T 276 | - |
|  | GTGATTTGTTAAGAAGATTAAT | G 277 | - |
|  | AGTAGTATTGTAAAGTATAAAGA | G 278 | - |
|  | TGATTGTTGTATAGTTATTGTGT | A 279 | - |
|  | GATTGTAGTTTAATGTTAAGAAT | G 280 | - |
|  | ATGAAATAAGAAATTGAGTAGAG | A 281 | - |
|  | TATGATGATATTTGTTGTATGTG | T 282 | - |
|  | TTTAGAGTTTGATTAGTATGTTT | G 283 | - |
|  | A ATAAGAGATTGTGATGAGAAAT | A 284 | - |
|  | A ATGAATAGAATAGAGAATGTAG | A 285 |  |
|  | GTAGTAGTAATTTGAATGTTTGA | A 286 | 47 |
|  | A GTGAGTAATTGATTGATTGTTA | A 287 | - |
|  | GAATAATGTTTAGTGTGTTTGAA | A 288 | - |
|  | A TATGAAAGTAGAGAAAGTGT A A A | T 289 | - |
|  | T T TGAA | G 290 | - |

Table I

TAGTTGAGTTTAAAGTTGAAAGAA TAAAGAGTGATGTAAATAGAAGTT TGTAGTGTTTAGAGTAAGTTATTA AGAGATTAATGTGTTGAAAGATTA GTAATAAGTTGTGAAAGAAGATTA GAGATGTTATAGATAATGAAAGAA TTTAGTTGATTGTTGAATAGAGTA ATTATTGAAAGTAGATGTTAGATG TTTATGTGTGATTGAGTGTTTAAT $T A T T T A G T T A G A T A G A T A G A G A G T$ ATGTGTTTATGTGAAAGATTTGTA ATAGTAATTAGAAGAGAAGAATGT TATGAGTGATTAGAATTGTATTTG TTAATGTATTGTTTAAAGAGTGTG ATAGAGAATTAAGAATTGTTTGAG GTTATAAGTAGAAATGTATAGAAG AGTAATTAGTTTGAAATGTGTAGT GAAAGATTATGATTGTAAAGTGAT GTAAGATTAGAAGTTAATGAAGAA GAGAATGTTGAATAAGAAGTAATT TTAAGAGTGTTTGAATAGTGTTTA ATAAAGAAAGAGTATGAGATTATG AGTTATTGATTGAAGATGAGAAAT GTTTGTGTTTGTATAAGTTGTTAA TTGTATGTGAGTTTAGATTAATGA TAGTTAAAGTATAGTTGTTTGAGT AAATTTGTGTTGAGATTTGTATAG TATTAGTGTTATGATAAAGAGAAG TATAAGAAGTAATTTGAGAAGAGT TAAGTTGAGATGTTTGTTTGATAA GTGTAGATTTATGAATTGAGTAAT TATAGAGAAGTGTTTAGTTGTATA ATAAAGAAGAATAGTTGTTGTGTA AGATTGAAATAGATTAGAAAGTTG GTTGTTATAAGAAATAGTTTGTTG AGAAATAGAGTAAGAGTGTTTAAA AGAGATAGTAGTAAATAGTTATTG AAATGATTGTGTAAGTTATGTATG AAGAAGTAAGAGAGAAATTTGAAT GTGTGTATTTAGTTGATAATTGAT ATTGTTGTTGTTGAGAAATGTATT AGATAAGTTAAAGTAAAGAGAATG TAGTTGAAGTTAGTTTAAGTGTTA AGTAAGAATGTAATATGATGATAG ATGAGATTGAAAGATTTATGAATG TGATTGAATTAGAGAGAATGTATA AGTTAGTAAGAGAATATAGTGAAT ATTAAGATTGTATAGTTAGTGATG GAGATAAAGAATTGAAATAGAAGA $A G A G T A A A T G T T A A G A A A G A A G T T$ AAAGTTTGTTATGTGTGAAGAATT ATTGTGTTTAAGAAATATGATGAG TATTGAAATGAGATGTATGTAGTT ATTTGTGTGATGTTTGAAATATGA TAAGATAATAGTGAGAGAAATTGA ATTTATGATTAGTGTAAGTGTTGT GATTAAGAATAAAGTGTGAAGAAT

291 292 293
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| Sequence SEQ | SEQ ID NO: | $\begin{array}{cl} \text { No. in } \\ \text { Ex } & 1 \end{array}$ |
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|  | 348 | - |
|  | 349 | - |
|  | 350 | - |
|  | 351 | - |
|  | 352 | - |
|  | 353 | - |
|  | 354 | - |
|  | 355 | - |
|  | 356 | - |
|  | 357 | 56 |
|  | 358 | - |
|  | 359 | - |
|  | 360 | - |
| A A A GTGTGTATATGTTAGAAAGTA | 361 | - |
|  | 362 | - |
|  | 363 | - |
|  | 364 | - |
| $T T T A A G T A T A A G A T T G T G T G A C A T$ | 365 | - |
|  | 366 | - |
|  | 367 | - |
|  | 368 | - |
|  | 369 | - |
|  | 370 | - |
|  | 371 | - |
|  | 372 | 60 |
|  | 373 | - |
| A A GTGTTT $T$ T A T GTAAGAGA A T G A A | 374 | 61 |
|  | 375 | - |
|  | 376 | - |
|  | 377 | - |
|  | 378 | - |
| $T \mathrm{~T} A \mathrm{~A} T \mathrm{~T}$ GAAGTGTTGTTTATTGAA | 379 | - |
|  | 380 | - |
|  | 381 | - |
|  | 382 | - |
| $T \mathrm{C}$ ATTTAGTATGTATTAGAGTTGA | 383 | - |
| TAAATAGAGATGAGAATAAGAGA | 384 | - |
|  | 385 | - |
| ATTTATGTAGTTGAGAGTGATAAA | 386 | - |
|  | 387 | - |
|  | 388 | - |
|  | 389 | - |
|  | 390 | - |
|  | 391 | - |
|  | 392 | - |
|  | 393 | - |
|  | 394 | - |
|  | - 395 | - |
|  | - 396 | - |
|  | - 397 | - |
|  | A 398 | - |
| ATTATAGAGGTTAGTTTAGAATGAG | G 399 | - |
| A A A G A TAGA A A T T G A G T G T A T G A T | - 400 | - |
| GTAGTTTTGTTAATGTTGTATAATM | G 401 | - |
|  | G 402 | 64 |
|  | G 403 | - |
|  | G 404 | - |

Table I



Table I


| ATGTTAAATGAGAGATTGTGATA | 576 | - |
| :---: | :---: | :---: |
| TAAATGTTGTGATTATTGTGAGAT | 577 | - |
| TAAGAATTGAAGTAAGAGTTATTG | 578 | - |
| A GAGATAGAATTAAGTTTGTTGAT | 579 | - |
| GAAGAATGTTAAGAAATATGTAAG | 580 | - |
| TATTTGTGATTAAGAAGTTGAGAA | 581 | - |
| A GTTAGAATTTGTGTAGTAGAATT | 582 | - |
| A AGTTTATTGTTGATGTTGTATTG | 583 | - |
| GAATGAGTTTAAGAGTTTATAGTA | 584 | - |
| AGTGAAGATTGTATGTAGTATAAA | 585 | - |
| A GTTGAAATGAGTATTAAGTAATG | 586 | - |
| ATGTGTTATTTGAGATGAGTAATT | 587 | - |
| A A A AGTGTTGTTGAAGTTGTTAT | 588 | - |
| GTAGAGAAAGATATATGTAGTTTA | 589 | - |
| GAGAGTATTTGATGAATGATTATA | 590 | - |
| GAGTATAAGTTTAGTGTATATTGA | 591 | - |
| ATAATGTGATTATTGATTGAGAGA | 592 | - |
| TTAGTTGTTATGTGAGAGTAATAA | 593 | - |
| AAATGAGTATATTGAATTGTGATG | 594 | - |
| AATTAGAAGTAAGTAGAGTTTAAG | 595 | 3 |
| $T \mathrm{GTAAGTTTAAAGTAAGAAATGTG}$ | 596 | 5 |
| GAAATGATAAGTTGATATAAGAAG | 597 | - |
| A ATGAGTAGTTTGTATTTGAGTTT | 598 | - |
| AGTGAATGTAAGATTATGTATTTG | 599 | 6 |
| GTAATTGAATTGAAAGATAAGTGT | 600 | 8 |
| TATGTTTAAGTAGTGAAATAGAGT | 601 | - |
| GTATTGAAATTGAATTAGAAGTAG | 602 | - |
| AATATGTAATGTAGTTGAAAGTGA | 603 | - |
| TGAATATTGAGAATTATGAGAGTT | 604 | - |
| TAGTGTAAATGATGAAGAAAGTAT | 605 | - |
| GTATGTGTAAAGAAATTTGATGTA | 606 | - |
| A ATTGTTTGAAAGTTTGTTGAGAA | 607 | - |
| AATTGTTTGAGTAGTATTAGTAGT | 608 | - |
| TAATTGAGTTTGAATAAGAGAGTT | 609 | - |
| TGTTGATTGTAAGTGTTTATTGTT | 610 | - |
| GAAATTTGTGAGTATGTATTTGAA | 611 | - |
| TAAGAATGAATGTGAAGTGAATAT | 612 | - |
| TAATGTGAAGTTTGTGAAAGATAT | 613 | - |
| TtGTATATGAAAGTAAGAAGAAGT | 614 | - |
| TAGAGAGAAGAAGAAATAAGAATA | 615 | - |
| ATTTGAAATGTTAATGAGAGAGAT | 616 | - |
| TTGTGTGTATATAGTATTAGAATG | 617 | - |
| ATTGTTAGTATTGATGTGAAGTTA | 618 | - |
| TGTTTGTATTTGAATGAAATGAAG | 619 | - |
| TGTTAGATTGTGTTAAATGTAGTT | 620 | - |
| TATAGAGTATTGTATAGAGAGAAA | 621 | - |
| A A A TAGTAAGAATGTAGTTGTTGA | 622 | - |
| TGAGTGTGATTTATGATTAAGTTA | 623 | - |
| AGAATTTGTTGTAGTGTTATGATT | 624 | - |
| GATTGAAGAAAGAAATAGTTGAA | 625 | - |
| GATAATAGAGAATAGTAGAGTTAA | 626 | - |
| GATTGAAATTTGTAGTTATAGTGA | 627 | - |
| GATTTAAGAAGATGAATAATGTAG | 628 | - |
| TTTGAGAGAAAGTAGAATAAGATA | 629 | - |
| GATTAAGAGTAAATGAGTATAAGA | 630 | - |
| TTTGATAGAATTGAAATTTGAGAG | 631 | - |
| TGAAGAAGAGTGTTATAAGATTTA | 632 | - |

Table I


Table I


Table I

|  |  | Ex |
| :---: | :---: | :---: |
| GTAGTAGATGATTAAGAAGATAAA | 747 | - |
| TTTAATGTGAAATTTGTTGTGAGT | 748 | - |
| GTAAAGAATTAGATAAAGAGTGAT | 749 | - |
| AATAGTTAAGTTTAAGAGTTGTGT | 750 | - |
| GTGTGATGTTTATAGATTTGTTAT | 751 | - |
| GTATAGTGTGATTAGATTTGTAAA | 752 | 49 |
| GTTGTAAGAAAGATATGTAAGAAA | 753 | - |
| ATATTAGATTGTAAAGAGAGTGAA | 754 | - |
| GAGTGATATTGAAATTAGATTGTA | 755 | - |
| TAAGAAGTTAAAGAAGAGAGTTTA | 756 | - |
| GATGTTAGATAAAGTTTAAGTAGT | 757 | - |
| GTGATTGTATGAGAAATGTTAAAT | 758 | - |
| TGATTATTGTAAGAAAGATTGAGA | 759 | - |
| AAGAATTGTGTAAGTTTATGAGTA | 760 | - |
| TTGTATTTAGAAGATTTGTAGATG | 761 | - |
| TATATGTTTGTGTAAGAAGAAATG | 762 | - |
| GATAATGTGTGAATTTGTGAATAA | 763 | - |
| TTAGAAATGTGAGATTTAAGAGTT | 764 | - |
| A GTGTAGAATTTGTATTTAGTTGT | 765 | - |
| TAGTTAAGATAGAGTAAATGATAG | 766 | - |
| GAAGTGATATTGTAAATTGATAAG | 767 | - |
| GTAATTGTGTTAGATTTAAGAAGT | 768 | - |
| TGATATTTGTGAATTGATAGTATG | 769 | - |
| A A GTAAAGAGATATAGTTAAGTTG | 770 | - |
| ATTAGTTAAGTTATTTGTGAGTGA | 771 | - |
| AGATGAAGTAGTTTATGAATTAGA | 772 | - |
| TGAGTTAGTTAAGTGATAGTTAAA | 773 | - |
| TTATTGTAGATTTAGAGAAGATGA | 774 | - |
| TATTTGTGTTTGTTGATTAGATAG | 775 | - |
| GTATAATGTGTGTGAAAGTTATAA | 776 | - |
| TATATGTTGAGTATAAAGAGAGAA | 777 | - |
| TTAGTTAGTTTAAAGATTGTGAGT | 778 | - |
| TTTAGAATAAGTGATGTGATGAAA | 779 | - |
| A GAGTAATGTGTAAATAGTTAGAT | 780 | - |
| TGTGATAAAGAGAAATTAGTTGTT | 781 | - |
| GAATTTAGTGAATGTTTGAGATTA | 782 | - |
| TGTGATGTGTAAGTATATGAAATT | 783 | - |
| TTGTGAATGATTAATGAATAGAAG | 784 | 51 |
| A A TGTTGTTTAGATTGAGAAAGTT | 785 | - |
| AGATTGTGTTAGTATTAGTATAAG | 786 | - |
| TTGATGTATTAGAAAGTTTATGTG | 787 | - |
| TATGATTGTGTGTTAGAGAATTTA | 788 | - |
| TAGTGTAGATATTTGATAGTTATG | 789 | 52 |
| AGTTTAATGTGTTTAGTTGTTATG | 790 | - |
| TGTGTAAAGTAGAAAGTAAAGATT | 791 | - |
| GTTATGATATAGTGAGTTGTTATT | 792 | 53 |
| TTTGATTGAATGTTAATAGTGTGT | 793 | - |
| AGAGTATTAGTAGTTATTGTAAGT | 794 | 54 |
| TAAGTAGAAAGAAGAAGATATTTG | 795 | - |
| AGAAAGAGAATTATGTAATGAAAG | 796 | - |
| TTAGATTTGTTAGTGTGATTTAAG | 797 | - |
| GATGATTAAGATATAGAGATAGTT | 798 | - |
| ATATTTGAGTGATTAAGAGTAATG | 799 | - |
| TGTATTGTGAGTTAAGTATAAGTT | 800 | - |
| A A T T TAGTAGAAAGTGTTGTGTTT | 801 | - |
| GTTAGAAGATTAAGTTGAATAATG | 802 | - |
| TAAAGTATGTGAGATGATTTATGT | 803 | - |

Table I


Table I

|  | Sequence | SEQ ID NO: | $\begin{gathered} \text { No. in } \\ \text { Ex } 1 \end{gathered}$ |
| :---: | :---: | :---: | :---: |
|  | GAAATGTGTATTTGTATGTTTAG | 861 | 59 |
|  | TTAAAGTTGATATGAAAGAAGTG | 862 | - |
|  | A TGTAGAGATTGTAGTGAATATT | 863 | 62 |
|  | TATTTGTTGAGTGTAAATGTGAT | 864 | - |
|  | TGTAATTGTGAATAATGTATGTG | - 865 | 63 |
|  | ATTTGTATAGAGATTAGTAAGTA | A 866 | - |
|  | A A ATTGTTGTTTAGAGAAAGAAG | -867 | - |
|  | A TGATGATGTATTTGTAAAGAGTA | A. 868 | - |
|  | A A GTATTTGTGTGATTGTGTAAA | A 869 | - |
|  | AGTGTTATGAAGAATAGTAAGAAT | T 870 | - |
|  | GTATGTAGAGATGAAAGAAATTA | A 871 | 65 |
|  | GTTTGTATTAGATAAATGAGTTGT | T 872 | - |
|  | TGATTTATGAGATTAAGAGAAAGA | A 873 | 70 |
|  | TTTGTGTGTTATTGTAATTGAGAT | T 874 | 70 |
|  | GATGTGTGATATGATTAAAGAAAT | T 875 |  |
|  | AGATTATAGATTTGTAGAGAAAGT | T 876 | - |
|  | GAAGAGTATGTAATAGTATTGTAT | T 877 | - |
|  | TTTGTAATGTTGTTGAGTTTAAGA | A 878 | - |
|  | A GTAAATAGTAGTATGAATAAGAG | G 879 | - |
|  | GAATGTTGAATTGAAATATGAGTT | T 880 |  |
|  | A GTAGTTAATTGATAGTAAGTTTG | G 8B1 | - |
|  | AGTGTAAAGAAATGAATGAATAAG | G 882 | - |
|  | TGTTAGATATTTGTGAAATGTGAA | A 883 |  |
|  | TGTATGTTGAGTTTGAATTGTTAT | T | - |
|  | $T G A G T G A A T T A G T T A T G T T G T T A T$ | $T \quad 885$ |  |
|  | GAAGAAAGAAATGAGAAAGATTAT | T 886 |  |
|  | T TAAGTAAGTTGTGTTGATATTAG | G 887 |  |
|  | ATGATGTGTTTGATTTGAATTGAA | A 888 | 72 |
|  | A A G TAAGTGAAATTGTTGTTTGAA | A 889 |  |
|  | ATGAAGTGTAAAGTTTGAAAGAA | A 890 |  |
|  | A GAGAGTAAGATAATTGTATAGT | A 891 |  |
|  | TTTATGAGATAGATGAAATAAGT | G 892 |  |
|  | A GAAA T TAGTAGTAATGATTTGT | G 893 |  |
|  | GATTTGAGATTGAATGAGAATAT | A 894 |  |
|  | GATTAGAAAGATGAATAAAGATG | A 895 |  |
|  | TAGATAGAAAGTATATGTTGTAG | T 896 |  |
|  | GAAGATAGTAAAGTAAAGTAAGT | 897 |  |
|  | AAATGTGTGTTTAGTAGTTGTAA | A 898 | 75 |
|  | T TGTTGAAGTAAGAGATGAATAA | A 899 |  |
|  | TATTTGAGAGAAAGAAAGAGTTT | A 900 |  |
|  | TATTTAGTGATGAATTTGTGATG | T 901 | - |
|  | TTATAGTGATGATGATAAGTTGA | T 902 |  |
|  | TAAAGATAATTGTAGAAAGTAGT | G 903 |  |
|  | GTTTAGTATTGATATTGTGTGTA | A 904 |  |
|  | GTGTTGTGAATAAGATTGAAATA | T 905 |  |
|  | AAAGAAAGTATAAAGTGAGATAG | A 906 | - |
|  | TATTTGTAAGAAGTGTAGATATT | G 907 |  |
|  | TAGAAGATGAAATTGTGATTTGT | T 908 |  |
|  | A TAATAGTAAGTGAATGATGAGA | T 909 |  |
|  | A A T G TGAATAAGATAAAGTGTGT | A 910 |  |
|  | A TTGAAGATAAAGATGTTGTTTA | G 911 |  |
|  | TGAAATAGAAGTGAGATTATAGT | A 912 | 76 |
|  | AGTTATTGTGAAAGAGTTTATGA | $\mathrm{T} \quad 913$ | - |
|  | A AATAGTAGTGATAGAGAAGATT | T 914 |  |
|  | AGTGTATGAAGTGTAATAAGATT | A 915 |  |
|  | $T G A T T A A G A T T G T G T A G T G T T A T$ | A 916 | - |
|  | AGTTTATGATATTTGTAGATGAG | T 917 |  |

Table I


Table I



| AGAGATGTTTATGTTGTGAATTA | 1089 | - |
| :---: | :---: | :---: |
|  | 1091 | - |
|  | 1092 | - |
| AAAGAGTGAATAGAAATAAGAGAA | 1093 | - |
| A ATAAAGTTATTGAGAGAGTTTAG | 1094 | - |
| A GTAGTGTTGTAGTTTAGTATATA | 1095 | - |
| GTAAGAATGTATTAGATATTTGTG | 1096 |  |
| GATAAATGTTTGATAAAGTAGTTG | 1097 | - |
| ATAGTATGTATGTGTGAAGATTTA | 1098 |  |
| ATGAATGTAGAGTGATTAGTTTAA | 1099 |  |
| GTAGTATTTAGTGATGTAAGAATA | 1100 |  |
| A GAATTGTATTGAAGAAGAATATG | 1101 |  |
| TTTATAGAATTGAGAGAAGTTAAG | 1102 |  |
| AAAGTAGTAGAGATTTGAGAATTA | 1103 | - |
| TTTAAAGAAAGTATTGTAAGAGTG | 1104 | - |
| AAATTGAGAAAGTGAATGAAGTTT | 1105 | - |
| A A GAAATAAGTATGATAGTAGTAG | 6 | - |
| ATTTGAATTGTATTGTAGTTTGTG | 1107 |  |
| A A GAGAATAATGTAGAGATATAAG | 1108 |  |
| TGTGTAATAGTTGTTAATGAGTAA | 1109 |  |
| TATAGTTGTAGTTTAGATGAATGT | 1110 | - |
| ATTGTGTTAGAATGATGTTAATAG | 1 |  |
| GTTTGTATAGTATTTGATTGATGT | 1112 |  |
| AGAGTAAAGTATGAGTTATGAATA | 1113 |  |
| GAAAGTTTAAGTGATGTATATTGT | 1114 | 96 |
| TTAAATGATAAAGAGTAGTGAAGT | 1115 |  |
| TTAAATGTGTGAGAAGATGAATAA | 1116 | - |
| ATt TGGTATAAAGTGAAGAAGAGAA | 1117 | 97 |
| TGATTAGTATTTGTGAAGAGATTT | 1118 | - |
| TTTGAATGAAATTGATGATAGATG | 1119 |  |
| A GAGTAAGATTAAGAATAAGAAAG | 1120 |  |
| ATTGAATTGAGAAGTGAAGTAAAT | 1121 |  |
| TTTAGAGAAGTATTGTTTGAAAGA | 1122 |  |
| TAAAGTGAAAGATTTGAAATGATG | 1123 |  |
| GAAAGTTAGAGAAATGTAGAAATT | 1124 |  |
| GTGAATAATGAAGAAGTTATGTTA | 1125 | 98 |
| TTGTGAATAAAGTAGATGTGTTAT | 1126 |  |
| TTATATGATATGAGTTTGTGTTGA | 1127 |  |
| TTGATTTGTGTGAGTATTAGTTAT | 1128 |  |
| AAAGTGATTAAGTTAGTTTGAGAT | 1129 |  |
| TTGTATTTGTATAATGTTGAAGAG | 1130 |  |
| GTTTTGAAATTAGTGTGAGAAATAT | 1131 |  |
| A ATGTTGAGATTGATAATGTTGAA | 1132 |  |
| TAGTAGTAGTATTGTTGTAATAAG | 1133 |  |
| GTTGTAATTTGAGTGTTAGTTATT | 1134 |  |
| TGAATATGATAGTTAGTAATTGTG | 1135 |  |
| TGATAGTATGTTTGTGATTAAAGA | 1136 |  |
| GATGTATAAAGAGTATGTTATAAG | 1137 |  |
| A GTGAGATTTAGAAGATGTTATTA | 1138 |  |
| ATGAGAATTTGTTAAAGAGAAAGT | 1139 |  |
| AAAGAATTAGTATGATAGATGAGA | 1140 | 99 |
| TAGAGTTGTATAGTTTATAGTTGA | 1141 | - |
| GTAGAATGATTGTTTAGAAGATTT | 1142 | - |
| GTTTATGTTTGAGAAGAGTTATTT | 1143 |  |
| TAGAAGTTTGAAAGTTATTGATTG | 1144 | - |
| GATGAAGAGTATTTGTTATATG | 1145 |  |

Table I

All references referred to in this specification are incorporated herein by reference.

The scope of protection sought for the invention described herein is defined by the appended claims. It will also be understood that any elements recited above or in the claims, can be combined with the elements of any claim. In particular, elements of a dependent claim can be combined with any element of a claim from which it depends, or with any other compatible element of the invention.

This application claims priority from United States Provisional Patent Application Nos. 60/263, 710 and 60/303, 799, filed January 25 , 2001 and July 10, 2001. Both of these documents are incorporated herein by reference.

## THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A composition comprising at least one hundred and fifty minimally crosshybridizing molecules for use as tags or tag complements wherein each molecule comprises an oligonucleotide comprising a sequence of nucleotide bases for which, under a defined set of conditions, the maximum degree of hybridization between said oligonucleotide and any complement of a different oligonucleotide does not exceed about $20 \%$ of the degree of hybridization between said oligonucleotide and a complement to said oligonucleotide, wherein for each said sequence there is at most six bases other than $G$ between every pair of neighbouring pairs of $G$ 's, and wherein the oligonucleotides are selected from SEQ ID NOs $1-1168$, or the complete complements of SEQ ID NOs 1-1168.
2. The composition of claim 1, wherein the number of G's in each said sequence does not exceed $L / 4$ where $L$ is the number of bases in said sequence.
3. The composition of claim 2 , wherein the number of $\mathrm{G}^{\prime}$ in each said sequence does not vary from the average number of G's in all of the sequences of the set by more than one.
4. The composition of claim 3, wherein the number of G's in each said sequence is the same as every other sequence of the set.
5. The composition of claim 3, wherein each said sequence is twenty-four bases in length and each said sequence contains 6 G's.
6. The composition of claim 1, wherein no oligonucleotide contains more than four contiguous bases that are identical to each other.
7. The composition of claim 1, wherein at the 5'-end of each said sequence at least one of the first, second, third, fourth, fifth, sixth and seventh bases of the sequence is a G.
8. The composition of claim 1 , wherein at the 3 '-end of each said sequence at least one of the first, second, third, fourth, fifth, sixth and seventh bases of the sequence is a G . 9. The composition of any one of claims 1 to 8 , wherein under said defined set of conditions, the maximum degree of hybridization between said oligonucleotide and any complement of a different oligonucleotide of the composition does not exceed about $6 \%$, and wherein said set of conditions results in a level of hybridisation that is the same
as the level of hybridisation obtained when hybridisation conditions include 0.2 M $\mathrm{NaCl}, 0.1 \mathrm{M}$ Tris, $0.08 \%$ Triton $\mathrm{X}-100, \mathrm{pH} 8.0$ at $37^{\circ} \mathrm{C}$, and the oligonucleotides are covalently linked to microparticles.
9. The composition of any one of claims 1 to 8 , wherein each said molecule is linked to a solid phase support so as to be distinguishable from a mixture of other said molecules by hybridization to its complement, and wherein each said molecule is linked to a defined location on said solid phase support, the defined location for each said molecule being different than the defined location for different other said molecules. 11. The composition of claim 10, wherein each said solid phase support is a microparticle and each said molecule is covalently linked to a different microparticle than each other different said molecule.
10. A composition comprising a plurality of minimally cross-hybridizing oligonucleotide tag complements, each oligonucleotide of each tag complement comprising:
(a) each oligonucleotide is free of either cytosine or guanosine residues;
(b) no two cytosine or guanosine residues are located adjacent each other in an oligonucleotide and any two cytosine or guanosine residues are separated by at most 6 non-cytosine or non-guanosine residues, respectively;
(c) the number of cytosine or guanosine residues in each oligonucleotide does not exceed $\mathrm{L} / 4$ where L is the number of bases in the oligonucleotide;
(d) the length of each oligonucleotide differs by no more than five bases from the average length of all oligonucleotides in the composition;
(e) each oligonucleotide does not contain 4 or more contiguous identical nucleotides;
(f) the number of guanosine or cytosine residues in each oligonucleotide does not vary from the average number of guanosine or cytosine residues in all other oligonucleotides of the composition by more than one; and
(g) when each oligonucleotide tag complement is exposed to hybridization conditions comprising $0.2 \mathrm{M} \mathrm{NaCl}, 0.1 \mathrm{M}$ Tris, $0.08 \%$ Triton $\mathrm{X}-100, \mathrm{pH} 8.0$ at $37^{\circ} \mathrm{C}$, the maximum degree of hybridization between the tag complement and a tag not fully complementary to the tag complement does not exceed $30 \%$ of the degree of hybridization between the tag complement and its fully complementary tag;
wherein the oligonucleotides are selected from SEQ ID NOs 1-1 168, or the complete complements of SEQ ID NOs 1-1168.
11. The composition of claim 12, wherein when each oligonucleotide contains a cytosine residue or a guanosine residue, each respective oligonucleotide contains either a cytosine or guanosine residue located within seven residues of an end of the oligonucleotide.
12. The composition of any one of claims 1 to 13 , wherein the length of each said sequence or each said oligonucleotide in the composition is the same.
13. The composition of any one of claims 1 to 13 , wherein each sequence or each oligonucleotide is between 10 and 50 bases or between 10 and 50 nucleotides in length, respectively.
14. The composition of claim 15, wherein each oligonucleotide comprises 24 residues and wherein each oligonucleotide contains either 6 guanosine or 6 cytosine residues.
15. The composition of claim 12 or 13 , wherein in element (g) the maximum degree of hybridization between the tag complement and a tag not fully complementary to the tag complement does not exceed $20 \%$ of the degree of hybridization between the tag complement and its fully complementary tag.
16. The composition of claim 17, wherein in element (g) the maximum degree of hybridization between the tag complement and a tag not fully complementary to the tag complement does not exceed $6 \%$ of the degree of hybridization between the tag complement and its fully complementary tag.
17. The composition of claim 12 or 13 , wherein the oligonucleotides are attached to a solid phase support.
18. The composition of claim 19 , wherein the support is a planar substrate comprising a plurality of spatially addressable regions.
19. The composition of claim 12 or 13 , wherein the oligonucleotides are covalently linked to microparticles, wherein the microparticles are spectrophotometrically unique and each unique microparticle has a different oligonucleotide attached thereto.
20. A kit for sorting and identifying polynucleotides, the kit comprising one or more solid phase supports each having one or more spatially discrete regions, each such region having a uniform population of substantially identical tag complements
covalently attached, and the tag complements each being selected from the set of oligonucleotides as defined in any of claims 1 to 18 .
21. A kit according to claim 22, wherein said one or more solid phase supports is a planar substrate and wherein said one or more spatially discrete regions is a plurality of spatially addressable regions.
22. A kit according to claim 22, wherein said one or more solid phase supports is a plurality of microparticles and each microparticle is spectrophotometrically unique from each other microparticle having a different oligonucleotide attached thereto.
23. A composition according to any one of claims 1 to 21 or a kit according to any one of claims 22 to 24 , substantially as herein described with reference to any one of the examples.

[^0]:    ABSTRACT

    A family of minimally cross-hybridizing nucleotide sequences, methods of 5 use, etc. A specific family of 116824 mers is described.

