

pre-selected 20-mer tags (e.g., using unique aptamers that bind to a specific protein). Nucleic acid aptamers capable of binding to virtually any protein of interest can be developed. See, for example, U.S. Pat. Nos. 5,270,163; 5,475,096; 5,567,588; 5,595,877; 5,637,459; 5,683,867, 5,705,337 and related patents. Reformatting approaches have the benefit of allowing different upstream assays on different target samples to be interrogated by the same chip hybridization platform. By decoupling the upstream biochemistry from the downstream detection process and executing the whole protocol for hundreds or thousands of probes in parallel, provides a very powerful analytic platform. The strategy of reformatting using hybridization tags, software used to generate the tags (publicly available), and genotyping assays using this approach is described in Hirschhorn, J., et al., "SBE-TAGS: An array-based method for efficient single-nucleotide polymorphism genotyping", *Proc. Natl. Acad. Sci.*, 97(22):12164-12169, 2000 and in Fan, et al., "Parallel genotyping of human SNPs using generic high-density oligonucleotide tag arrays", *Genome Res.* 2000 Jun;10(6):853-60. A set of standard hybridization tags is available at <http://waldo.wi.mit.edu/publications/SBE-TAGS/>.

[0183] VI. Assays

[0184] The magnetic chip and bead technology can support any of a wide variety of reactions and assays. These reactions and assays may include essentially any of the reactions and assays conventionally performed using molecules attached to beads and those performed using conventional DNA arrays. For example, nucleic acid hybridization assays, enzymatic reactions, antigen-antibody reactions, assays for protein-protein interactions, assays for interaction of small molecules with nucleic acids and/or proteins, screening of combinatorial chemical libraries, etc., can all be performed using bead-based approaches.

[0185] The magnetic chip of the present invention may find particular use in reactions involving nucleic acids and in assays for detecting nucleic acid interactions. A large and varied assortment of such reactions and assays are available, a number of which are described, for example, in WO0048000 and in WO0063437 and in patents and publications referenced therein. Reactions include various ligation and polymerization reactions including amplification reactions such as polymerase chain reaction (PCR), oligonucleotide ligase amplification (OLA), cycling probe technology (CPT), strand displacement assay (SDA), transcription mediated amplification (TDA), nucleic acid sequence based amplification (NASBA), rolling circle amplification (RCA), and invasive cleavage technology. Assays include, but are not limited to, genotyping assays such as simple or competitive hybridization, allelic PCR, OLA which may employ a ligation chain reaction (LCR), single base extension (SBE), allele-specific primer extension (ASPE), exonuclease assays such as Taqman, invasive cleavage, and/or a combination of any of the foregoing. Additional examples of assays that can be performed in the context of the present invention are found in, Steemers, F., et al., "Screening unlabeled DNA targets with randomly ordered fiber-optic gene arrays", *Nat Biotechnology*, 18:91-94, 2000, describing use of bead-coupled probes incorporating molecular beacons for detection of mutations in genes of the cystic fibrosis transmembrane conductor region.

[0186] Example 3 describes a DNA hybridization assay in which oligonucleotides were attached to magnetic beads (via

streptavidin-biotin linkage), which were then incubated with complementary oligonucleotides labeled with the fluorescent molecule Cy3. **FIG. 13** shows a fluorescence image obtained after performing the hybridization off-chip and then arraying the beads on a magnetic chip of the invention.

[0187] Assays involving RNA, e.g., measurements of mRNA abundance may conveniently be performed using the magnetic chip, as is commonly done using conventional cDNA or oligonucleotide arrays. Another example of an assay involving RNA that can be performed in the context of the present invention is described in Brenner, S., et al., "Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays", *Nat Biotechnol*, Jun;18(6):630-4, 2000, describing a method for determining mRNA abundance using cDNA libraries cloned onto the surfaces of microbeads.

[0188] The magnetic chip may also be used to form randomly ordered protein arrays, e.g., antibody arrays. The use of antibody arrays is described, for example, in Haab, B., et al., "Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions", *Genome Biol.* 2001;2(2), 2001. Other types of protein arrays are known in the art. Antibody-based assays such as enzyme-linked immunosorbent assays (ELISA) may also be performed on beads and thus employed in the context of the present invention.

[0189] In order to perform many of the above assays it is necessary to couple one or more molecules to a magnetic particle. Any of a wide variety of coupling methods may be employed. Coupling can be covalent or noncovalent. One of ordinary skill in the art will readily be able to select and apply an appropriate method (e.g., depending upon the type of molecule to be coupled). Coupling can be performed using chemical or affinity capture, cross-linking, electrostatic attachment, etc. In affinity capture, the bead is derivatized with one member of a binding pair while the molecule to be captured is derivatized with the other. Appropriate binding pairs include, but are not limited to, (i) biotin and streptavidin or derivatives thereof; (ii) complementary or substantially complementary nucleic acids (e.g., oligo-dT and poly-A regions of mRNA); (iii) protein A, G, or L and Ig; (iv) carbohydrate-lectin pairs; (v) hapten-antibody pairs, (vi) aminealdehyde pairs, etc. Molecules may be attached to beads via linkers, of which a large number are known in the art. See, for example, Pierce Chemical Co. Catalog, Pierce Chemical Co., Rockford Ill. See also, Hermanson, G., *Bioconjugate Techniques*, Academic Press, San Diego, 1996. Examples of linkers include sulfhydryl reactive linkers such as maleimides, etc. The surface of beads may be derivatized with various functional groups to facilitate attachment of molecules. Such functional groups include amino groups, carboxyl groups, aldehydes, amides, chloromethyl groups, hydrazides, hydroxyl groups, and sulfonates. Methods for attaching nucleotides and/or nucleic acids to the surfaces of derivatized microbeads, e.g., via a base-labile group, and methods for attaching polypeptides, e.g., via amino groups are also well known in the art. Molecules such as nucleic acids or polypeptides may also be synthesized directly on the bead.

[0190] As noted above, performing assays on microbeads has a number of advantages. For example, in multistep assays it is convenient to add and remove reagents when