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The present invention concerns methods and compositions for introducing miRNA activity or function into cells using synthetic nucleic acid molecules. Moreover, the present invention concerns methods and compositions for identifying miRNAs with specific cellular functions that are relevant to therapeutic, diagnostic, and prognostic applications wherein synthetic miRNAs and/or miRNA inhibitors are used in library screening assays.

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METHODS AND COMPOSITIONS INVOLVING MIRNA AND MIRNA INHIBITOR MOLECULES

ABSTRACT

The present invention concerns methods and compositions for introducing miRNA activity or function into cells using synthetic nucleic acid molecules. Moreover, the present invention concerns methods and compositions for identifying miRNAs with specific cellular functions that are relevant to therapeutic, diagnostic, and prognostic applications wherein synthetic miRNAs and/or miRNA inhibitors are used in library screening assays.

METHODS AND COMPOSITIONS INVOLVING MIRNA AND MIRNA INHIBITOR MOLECULES

This application is a divisional of application serial number 2,587,189, which is the national phase application derived from international application PCT/US2005/041162 filed internationally on November 14, 2005, which claims convention priority from United States patent applications 60/627,171 filed November 12, 2004, 60/649,634 filed February 3, 2005, and 60/683,736 filed May 23, 2005.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the field of molecular biology. More particularly, it concerns methods and compositions involving nucleic acid molecules that simulate microRNA (miRNAs) and that inhibit miRNAs. Methods and compositions involving synthetic miRNAs and miRNA inhibitor molecules are described. In addition, methods and compositions for identifying miRNAs that contribute to cellular processes are also described. In addition, the identification of miRNAs that contribute to cellular processes provides targets for therapeutic intervention as well as diagnostic and/or prognostic analysis.

2. Description of the Related Art

In 2001, several groups used a novel cloning method to isolate and identify a large group of "microRNAs" (miRNAs) from *C. elegans*, *Drosophila*, and humans (Lagos-Quintana *et al.*, 2001; Lau *et al.*, 2001; Lee and Ambros, 2001). Several hundreds of miRNAs have been identified in plants and animals—including humans—which do not appear to have endogenous siRNAs. Thus, while similar to siRNAs, miRNAs are nonetheless distinct.

miRNAs thus far observed have been approximately 21-22 nucleotides in length and they arise from longer precursors, which are transcribed from non-protein-encoding genes. *See* review of Carrington *et al.* (2003). The precursors form structures that fold back on each other in self-complementary regions; they are then processed by the nuclease Dicer in animals or DCL1 in plants. miRNA molecules interrupt translation through precise or imprecise base-pairing with their targets.

miRNAs seem to be involved in gene regulation. Some miRNAs, including *lin-4* and *let-7*, inhibit protein synthesis by binding to partially complementary 3′ untranslated regions (3′ UTRs) of target mRNAs. Others, including the *Scarecrow* miRNA found in plants, function like siRNA and bind to perfectly complementary mRNA sequences to destroy the target transcript (Grishok *et al.*, 2001).

Research on microRNAs is increasing as scientists are beginning to appreciate the broad role that these molecules play in the regulation of eukaryotic gene expression. The two best understood miRNAs, *lin-4* and *let-7*, regulate developmental timing in *C. elegans* by regulating the translation of a family of key mRNAs (reviewed in Pasquinelli, 2002). Several hundred miRNAs have been identified in *C. elegans*, *Drosophila*, mouse, and humans. As would be expected for molecules that regulate gene expression, miRNA levels have been shown to vary between tissues and developmental states. In addition, one study shows a strong correlation between reduced expression of two miRNAs and chronic lymphocytic leukemia, providing a possible link between miRNAs and cancer (Calin, 2002). Although the field is still young, there is speculation that miRNAs could be as important as transcription factors in regulating gene expression in higher eukaryotes.

There are a few examples of miRNAs that play critical roles in cell differentiation, early development, and cellular processes like apoptosis and fat metabolism. *lin-4* and *let-7* both regulate passage from one larval state to another during C. elegans development (Ambros, 2003). *mir-14* and *bantam* are *drosophila* miRNAs that regulate cell death, apparently by regulating the expression of genes involved in apoptosis (Brennecke *et al.*, 2003, Xu *et al.*, 2003). MiR14 has also been implicated in fat metabolism (Xu *et al.*, 2003). *Lsy-6* and *miR-273* are C. elegans miRNAs that regulate asymmetry in chemosensory neurons (Chang *et al.*, 2004). Another animal miRNA that regulates cell differentiation is miR-181, which guides hematopoietic cell differentiation (Chen *et al.*, 2004). These molecules represent the full range of animal miRNAs with known functions. Enhanced understanding of the functions of miRNAs will undoubtedly reveal regulatory networks that contribute to normal development, differentiation, inter- and intra-cellular communication, cell cycle, angiogenesis, apoptosis, and many other cellular processes. Given their important roles in many biological functions, it is likely that miRNAs will offer important points for therapeutic intervention or diagnostic analysis.

Characterizing the functions of biomolecules like miRNAs often involves introducing the molecules into cells or removing the molecules from cells and measuring the result. If introducing a miRNA into cells results in apoptosis, then the miRNA undoubtedly participates in an apoptotic pathway. Methods for introducing and removing miRNAs from cells have been described. Two recent publications describe antisense molecules that can be used to inhibit the activity of specific miRNAs (Meister *et al.*, 2004; Hutvagner *et al.*, 2004). Another publication describes the use of plasmids that are transcribed by endogenous RNA polymerases and yield specific miRNAs when transfected into cells (Zeng *et al.*, 2002). These two reagent sets have been used to evaluate single miRNAs.

A limitation of the plasmid-based miRNA expression system is that the transfection efficiencies for plasmids tend to be very low, with only approximately 50% of cells expressing RNA from the plasmid in cells that are easy to transfect. Transfection efficiencies for plasmids in primary cells are much lower, with fewer than 10% of cells typically expressing the desired RNA. Therefore, there is a need for alternative compositions and methods for introducing miRNA molecules into cells so that they can be characterized and studied.

SUMMARY OF THE INVENTION

Certain exemplary embodiments provide a synthetic RNA molecule comprising: a) a first 17-25 residue oligonucleotide comprising a sequence that is 80-100% identical to a mature microRNA sequence, b) a second 17-25 residue oligonucleotide comprising a sequence that is 60-100% identical to a sequence complementary to the mature microRNA sequence, and c) at least one of i) a replacement group for the phosphate or the hydroxyl of the nucleotide at the 5' end of the second oligonucleotide, ii) one or more sugar modifications in the first or last 1-6 residues of the second oligonucleotide, and iii) a noncomplementarity between one or more nucleotides in the last 1-5 residues at the 3' end of the second oligonucleotide and the corresponding nucleotides of the first oligonucleotide.

Other exemplary embodiments provide a synthetic RNA molecule comprising: a) a first 17-25 residue oligonucleotide comprising a sequence that is 80-100% identical to a mature microRNA sequence, b) a second 17-25 residue oligonucleotide comprising a sequence that is 60-100% identical to a sequence complementary to the mature microRNA sequence, and c) at least one modification to the second oligonucleotide that enhances the uptake of the first oligonucleotide.

The present invention is based on the inventors' studies regarding the introduction into cells of one or more nucleic acids that function like miRNA or inhibit the activities of one or more miRNAs in cells to characterize their roles in various biological processes. The invention concerns nucleic acids that perform the activities of endogenous miRNAs when introduced into cells. These nucleic acids are synthetic miRNA in some embodiments. The invention further concerns a library of synthetic miRNAs specific to a variety of known miRNAs that can be used to introduce sequentially or in combination one or more miRNAs into cells *in vitro* or *in vivo* for the purpose of identifying miRNAs that participate in cellular processes. The invention further involves a library of sequence-specific miRNA inhibitors that can be used to inhibit sequentially or in combination the activities of one or more miRNAs in cells. The two libraries of miRNA-specific reagents are used to introduce or eliminate specific miRNAs or combinations of miRNAs to define the roles of miRNAs in cells.

The term “miRNA” is used according to its ordinary and plain meaning and refers to a microRNA molecule found in eukaryotes that is involved in RNA-based gene regulation. *See, e.g.,* Carrington *et al.*, 2003. The term will be used to refer to the single-stranded RNA molecule processed from a precursor. Individual miRNAs have been identified and sequenced in different organisms, and they have been given names. Names of miRNAs and their sequences are provided herein. Additionally, other miRNAs are known to those of skill in the art and can be readily implemented in embodiments of the invention. The methods and compositions should not be limited to miRNAs identified in the application, as they are provided as examples, not necessarily as limitations of the invention.

The present invention concerns, in some embodiments of the invention, short nucleic acid molecules that function as miRNAs or as inhibitors of miRNA in a cell. The term “short” refers to a length of a single polynucleotide that is 150 nucleotides or fewer. The nucleic acid molecules are synthetic. The term “synthetic” means the nucleic acid molecule is isolated and not identical in sequence (the entire sequence) and/or chemical structure to a naturally-occurring nucleic acid molecule, such as an endogenous precursor miRNA molecule. While in some embodiments, nucleic acids of the invention do not have an *entire* sequence that is identical to a sequence of a naturally-occurring nucleic acid, such molecules may encompass all or part of a naturally-occurring sequence. It is contemplated, however, that a synthetic nucleic acid administered to a cell may subsequently be modified or altered in the cell such that its structure or sequence is the same as non-synthetic or naturally occuring nucleic acid, such as a mature miRNA sequence. For example, a synthetic nucleic acid may have a sequence that differs from the sequence of a precursor miRNA, but that sequence may be altered once in a cell to be the same as an endogenous, processed miRNA. The term “isolated” means that the nucleic acid molecules of the invention are initially separated from different (in terms of sequence or structure) and unwanted nucleic acid molecules such that a population of isolated nucleic acids is at least about 90% homogenous, and may be at least about 95, 96, 97, 98, 99, or 100% homogenous with respect to other polynucleotide molecules. In many embodiments of the invention, a nucleic acid is isolated by virtue of it having been synthesized *in vitro* separate from endogenous nucleic acids in a cell. It will be understood, however, that isolated nucleic acids may be subsequently mixed or pooled together.

Of course, it is understood that a "synthetic nucleic acid" of the invention means that the nucleic acid does not have a chemical structure or sequence of a naturally occuring nucleic acid. Consequently, it will be understood that the term "synthetic miRNA" refers to a "synthetic nucleic acid" that functions in a cell or under physiological conditions as a naturally occuring miRNA.

While many of the embodiments of the invention involve synthetic miRNAs or synthetic nucleic acids, in some embodiments of the invention, the nucleic acid molecule(s) need not be "synthetic." In certain embodiments, a non-synthetic miRNA employed in methods and compositions of the invention may have the entire sequence and structure of a naturally occurring miRNA precursor or the mature miRNA. For example, non-synthetic miRNAs used in methods and compositions of the invention may not have one or more modified nucleotides or nucleotide analogs. In these embodiments, the non-synthetic miRNA may or may not be recombinantly produced. In particular embodiments, the nucleic acid in methods and/or compositions of the invention is specifically a synthetic miRNA and not a non-synthetic miRNA (that is, not an miRNA that qualifies as "synthetic"); though in other embodiments, the invention specifically involves a non-synthetic miRNA and not a synthetic miRNA. Any embodiments discussed with respect to the use of synthetic miRNAs can be applied with respect to non-synthetic miRNAs, and vice versa.

It will be understood that the term "naturally occurring" refers to something found in an organism without any intervention by a person; it could refer to a naturally-occurring wildtype or mutant molecule. In some embodiments a synthetic miRNA molecule does not have the sequence of a naturally occurring miRNA molecule. In other embodiments, a synthetic miRNA molecule may have the sequence of a naturally occurring miRNA molecule, but the chemical structure of the molecule, particularly in the part unrelated specifically to the precise sequence (non-sequence chemical structure) differs from chemical structure of the naturally occurring miRNA molecule with that sequence. In some cases, the synthetic miRNA has both a sequence and non-sequence chemical structure that are not found in a naturally-occurring miRNA. Moreover, the sequence of the synthetic molecules will identify which miRNA is effectively being provided or inhibited; the endogenous miRNA will be referred to as the "corresponding miRNA." Corresponding miRNA sequences that can be used in the context of the invention include, but are not limited to, those sequences in SEQ ID NOs: 1-593 and those miRNAs listed in the appendix.. In addition synthetic nucleic acids of the invention may include SEQ ID NOs:594-703 as well as any other miRNA sequence, miRNA precursor sequence, or any sequence complementary thereof. In some embodiments, the sequence is or is derived from a probe sequence identified in the appendix to target the particular miRNA (or set of miRNAs) that can be used with that probe sequence.

Synthetic miRNA of the invention are RNA or RNA analogs in some embodiments of the invention. MiRNA inhibitors may be DNA or RNA, or analogs thereof. miRNA and miRNA inhibitors of the invention are collectively referred to as "synthetic nucleic acids."

In some embodiments, there is a synthetic miRNA having a length of between 17 and 130 residues. The present invention concerns synthetic miRNA molecules that are, are at least, or are at most 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, or 130 residues in length, or any range derivable therein.

In certain embodiments, synthetic miRNA have a) an "miRNA region" whose sequence from 5′ to 3′ is identical to a mature miRNA sequence, and b) a "complementary region" whose sequence from 5′ to 3′ is between 60% and 100% complementary to the miRNA sequence. In certain embodiments, these synthetic miRNA are also isolated, as defined above. The term "miRNA region" refers to a region on the synthetic miRNA that is at least 90% identical to the entire sequence of a mature, naturally occurring miRNA sequence. In certain embodiments, the miRNA region is or is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or 100% identical to the sequence of a naturally-occurring miRNA.

The term "complementary region" refers to a region of a synthetic miRNA that is or is at least 60% complementary to the mature, naturally occurring miRNA sequence that the miRNA region is identical to. The complementary region is or is at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or 100% complementary, or any range derivable therein. With single polynucleotide sequences, there is a hairpin loop structure as a result of chemical bonding between the miRNA region and the complementary region. In other embodiments, the complementary region is on a different nucleic acid molecule than the miRNA region, in which case the complementary region is on the complementary strand and the miRNA region is on the active strand.

In other embodiments of the invention, there are synthetic nucleic acids that are miRNA inhibitors. An miRNA inhibitor is between about 17 to 25 nucleotides in length and comprises a 5′ to 3′ sequence that is at least 90% complementary to the 5′ to 3′ sequence of a mature miRNA. In certain embodiments, an miRNA inhibitor molecule is 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length, or any range derivable therein. Moreover, an miRNA inhibitor has a sequence (from 5′ to 3′) that is or is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or 100% complementary, or any range derivable therein, to the 5' to 3' sequence of a mature miRNA, particularly a mature, naturally occurring miRNA. Probe sequences for miRNAs are disclosed in the appendix. While they have more sequence than an miRNA inhibitor, one of skill in the art could use that portion of the probe sequence that is complementary to the sequence of a mature miRNA as the sequence for an miRNA inhibitor. Table 1 indicates what the mature sequence of an miRNA is. Moreover, that portion of the probe sequence can be altered so that it is still 90% complementary to the sequence of a mature miRNA.

In some embodiments, of the invention, a synthetic miRNA contains one or more design elements. These design elements include, but are not limited to: i) a replacement group for the phosphate or hydroxyl of the nucleotide at the 5′ terminus of the complementary region; ii) one or more sugar modifications in the first or last 1 to 6 residues of the complementary region; or, iii) noncomplementarity between one or more nucleotides in the last 1 to 5 residues at the 3′ end of the complementary region and the corresponding nucleotides of the miRNA region.

In certain embodiments, a synthetic miRNA has a nucleotide at its 5′ end of the complementary region in which the phosphate and/or hydroxyl group has been replaced with another chemical group (referred to as the "replacement design"). In some cases, the phosphate group is replaced, while in others, the hydroxyl group has been replaced. In particular embodiments, the replacement group is biotin, an amine group, a lower alkylamine group, an acetyl group, 2'O-Me (2'oxygen-methyl), DMTO (4,4'-dimethoxytrityl with oxygen), fluoroscein, a thiol, or acridine, though other replacement groups are well known to those of skill in the art and can be used as well. This design element can also be used with an miRNA inhibitor.

Additional embodiments concern a synthetic miRNA having one or more sugar modifications in the first or last 1 to 6 residues of the complementary region (referred to as the "sugar replacement design"). In certain cases, there is one or more sugar modifications in the first 1, 2, 3, 4, 5, 6 or more residues of the complementary region, or any range derivable therein. In additional cases, there is one or more sugar modifications in the last 1, 2, 3, 4, 5, 6 or more residues of the complementary region, or any range derivable therein, have a sugar modification. It will be understood that the terms "first" and "last" are with respect to the order of residues from the 5′ end to the 3′ end of the region. In particular embodiments, the sugar modification is a 2'O-Me modification. In further embodiments, there is one or more sugar modifications in the first or last 2 to 4 residues of the complementary region or the first or last 4 to 6 residues of the complementary region. This design element can also be used with an miRNA inhibitor. Thus, an miRNA inhibitor can have this design element and/or a replacement group on the nucleotide at the 5' terminus, as discussed above.

In other embodiments of the invention, there is a synthetic miRNA in which one or more nucleotides in the last 1 to 5 residues at the 3′ end of the complementary region are not complementary to the corresponding nucleotides of the miRNA region ("noncomplementarity") (referred to as the "noncomplementarity design"). The noncomplementarity may be in the last 1, 2, 3, 4, and/or 5 residues of the complementary miRNA. In certain embodiments, there is noncomplementarity with at least 2 nucleotides in the complementary region.

It is contemplated that synthetic miRNA of the invention have one or more of the replacement, sugar modification, or noncomplementarity designs. In certain cases, synthetic RNA molecules have two of them, while in others these molecules have all three designs in place.

The miRNA region and the complementary region may be on the same or separate polynucleotides. In cases in which they are contained on or in the same polynucleotide, the miRNA molecule will be considered a single polynucleotide. In embodiments in which the different regions are on separate polynucleotides, the synthetic miRNA will be considered to be comprised of two polynucleotides.

When the RNA molecule is a single polynucleotide, there is a linker region between the miRNA region and the complementary region. In some embodiments, the single polynucleotide is capable of forming a hairpin loop structure as a result of bonding between the miRNA region and the complementary region. The linker constitutes the hairpin loop. It is contemplated that in some embodiments, the linker region is, is at least, or is at most 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 residues in length, or any range derivable therein. In certain embodiments, the linker is between 3 and 30 residues (inclusive) in length.

In addition to having an miRNA region and a complementary region, there may be flanking sequences as well at either the 5′ or 3′ end of the region. In some embodiments, there is or is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 nucleotides or more, or any range derivable therein, flanking one or both sides of these regions.

The present invention also concerns a collection of synthetic nucleic acid molecules, referred to as a library. A collection may contain, contain at least or contain at most 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550 or more different types (by structure and/or sequence) of nucleic acids. Libraries may contain synthetic miRNAs and/or miRNA inhibitors.

Embodiments involving libraries and methods of using nucleic acids of the invention may be applied to miRNA and miRNA inhibitors. Thus, any embodiment discussed with respect to nucleic acids of the invention may generally be applicable to miRNA and miRNA inhibitor molecules, and vice versa. Moreover, embodiments discussed with respect to miRNA may be applied to miRNA inhibitors and vice versa.

The present invention also concerns methods of characterizing an miRNA activity or function in a cell. In some embodiments, a method comprises: a) introducing into one or more cells a synthetic miRNA molecule; and b) comparing one or more characteristics of cell(s) having the RNA molecule with cells in which the synthetic miRNA molecule has not been introduced. In certain embodiments, the cells with the synthetic miRNA may be compared to cells in which a different molecule was introduced (such as a negative control that does not include an miRNA region or has an miRNA region for a different miRNA). It is contemplated that the compared cells need not be evaluated at the same time. In fact, the comparison cells need not have been cultured at the same time; one may refer to a report or previous observation.

Other methods include reducing or eliminating activity of one or more miRNAs from a cell comprising: a) introducing into a cell an miRNA inhibitor. In certain embodiment, methods also include comparing one or more characteristics of a cell having the miRNA inhibitor with a cell not having the miRNA inhibitor.

The synthetic nucleic acids discussed above and herein can be used in methods of the invention. Thus, in certain embodiments, the methods involve synthetic nucleic acids with the different designs in them.

Characteristics of cells that may be evaluated are not limited. They include the following characteristics and characteristics associated with the following: cell proliferation, mitotic index, cell cycle, apoptosis, motility, adhesion, signal transduction, protein localization, gene expression, RNA localization, cell division, DNA replication, post-translational modification, differentiation, de-differentiation, transcriptional activation, protein activation, angiogenesis, metabolism (energy production and/or consumption), protein degradation, chromatin condensation, microtubule production, DNA replication, recombination, and DNA repair functions. It is contemplated that these characteristics may be relevant globally to the cell (for example, overall protein production reduced) or to individual species in the cell (for example, induction of a specific protein(s)).

It is contemplated that this method may be applied with respect to a variety of different synthetic and/or nonsynthetic miRNAs in separate or the same cells. In some cases, the following numbers of different synthetic miRNA molecules may be introduced into different cells: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300 or more, or any range derivable therein (or at least or at most these numbers). The invention is not limited by cell type. It is contemplated that any cell expressing miRNA or any cell having a characteristic altered by an miRNA is amenable to the methods and compositions of the invention. Use of two or more miRNAs may be combined in a single pharmaceutical composition as a cocktail or may be used in any therapeutic, diagnostic or prognostic method of the invention. It is contemplated that methods of the invention may involve, involve at least, or involve at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300 or more, or any range derivable therein, nucleic acid molecules corresponding to different miRNAs. Such nucleic acid molecules include synthetic miRNAs molecules, nonsynthetic miRNA molecules, and miRNA inhibitors.

In some embodiments, it may be useful to know whether a cell expresses a particular miRNA endogenously or whether such expression is affected under particular conditions or when it is in a particular disease state. Thus, in some embodiments of the invention, methods include assaying the cell for the presence of the miRNA that is effectively being introduced by the synthetic miRNA molecule or inhibited by an miRNA inhibitor. Consequently, in some embodiments, methods include a step of generating an miRNA profile for a sample. The term "miRNA profile" refers to a set of data regarding the expression pattern for a plurality of miRNAs in the sample; it is contemplated that the miRNA profile can be obtained using an miRNA array. In some embodiments of the invention, an miRNA profile is generated by steps that include: a) labeling miRNA in the sample; b) hybridizing the miRNA to an miRNA array; and, c) determining miRNA hybridization to the array, wherein an miRNA profile is generated.

Additionally, a cell that is introduced with a synthetic miRNA or an miRNA inhibitor may be subsequently evaluated or assayed for the amount of endogenous or exogenous miRNA or miRNA inhibitor. Any cell type is contemplated for use with the invention. The cell may be from or in a mammal, such as a monkey, horse, cow, pig, sheep, dog, cat, rabbit, mouse, rat, or human.

In other methods of the invention, a step of synthesizing or obtaining the synthetic RNA molecule is included.

In additional embodiments, the synthetic nucleic acid is introduced into the cell by calcium phosphate transfection, lipid transfection, electroporation, microinjection, or injection. In addition, a cell may be in a subject, which may be a patient or an animal model. In this case, synthetic nucleic acids can be administered to the subject or patient using modes of administration that are well known to those of skill in the art, particularly for therapeutic applications. It is particularly contemplated that a patient is human or any other mammal or animal having miRNA.

The present invention also concerns inducing certain cellular characteristics by providing to a cell a particular nucleic acid, such as a specific synthetic miRNA molecule or a synthetic miRNA inhibitor molecule. However, in methods of the invention, the miRNA molecule or miRNA inhibitor need not be synthetic. They may have a sequence that is identical to a naturally occurring miRNA or they may not have any design modifications. In certain embodiments, the miRNA molecule and/or an miRNA inhibitor are synthetic, as discussed above.

The particular nucleic acid molecule provided to the cell is understood to correspond to a particular miRNA in the cell, and thus, the miRNA in the cell is referred to as the "corresponding miRNA." In situations in which a named miRNA molecule is introduced into a cell, the corresponding miRNA will be understood to be the induced miRNA. It is contemplated, however, that the miRNA molecule provided introduced into a cell is not a mature miRNA but is capable of becoming a mature miRNA under the appropriate physiological conditions. In cases in which a particular corresponding miRNA is being inhibited by a miRNA inhibitor, the particular miRNA will be referred to as the targeted miRNA. It is contemplated that multiple corresponding miRNAs may be involved. In particular embodiments, more than one miRNA molecule is introduced into a cell. Moreover, in other embodiments, more than one miRNA inhibitor is introduced into a cell. Furthermore, a combination of miRNA molecule(s) and miRNA inhibitor(s) may be introduced into a cell.

Methods include identifying a cell or patient in need of inducing those cellular characteristics. Also, it will be understood that an amount of a synthetic nucleic acid that is provided to a cell or organism is an "effective amount," which refers to an amount needed to achieve a desired goal, such as inducing a particular cellular characteristic(s).

In certain embodiments of the methods include providing or introducing to a cell a nucleic acid molecule corresponding to a mature miRNA in the cell in an amount effective to achieve a desired physiological result. Such methods are disclosed herein. Moreover, methods of the invention involve diagnosing a patient based on an miRNA expression profile. In certain embodiments, the elevation or reduction in the level of expression of a particular miRNA in a cell is correlated with a disease state compared to the expression level of that miRNA in a normal cell. This correlation allows for diagnostic methods to be carried out when that the expression level of an miRNA is measured in a biological sample being assessed and then compared to the expression level of a normal cell.

In these different methods, the corresponding miRNA involved in the method may be one or more of at least the following: Let 7a, let 7a-1, let 7b, let 7b-1, let-7c, let-7d, let 7g, miR-1, miR-1-d, miR-1-2, miR-9, miR-10a, miR-10b, miR-15a, miR-16, miR-17, miR-17-3p, miR-18, miR-19a, miR-20, miR-21, miR-22, miR-23, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-27a, miR-28, miR-29a, miR-29b, miR-30a-3p, miR-30a, miR-30e-5p, miR-31, miR-32, miR-34a, miR-92, miR-93, miR-95, miR-96, miR-98, miR-99a, miR-100, miR-101, miR-105, miR-106, miR-107, miR-108, miR-122, miR-124, miR-125, miR-125b, miR-126, miR-127, miR-128, miR-129, miR-130, miR-130a, miR-133, miR-133a, miR-133a-2, miR-133b, miR-134, miR-135, miR-137, miR-138, miR-139, miR-140, miR-141, miR-142, miR-143, miR-145, miR-147, miR-148, miR-149, miR-150, miR-152, miR-153, miR-154, miR-155, miR-181, miR-182, miR-183, miR-184, miR-186, miR-187, miR-188, miR-190, miR-191, miR-192, miR-193, miR-194, miR-195, miR-196, miR-197, miR-198, miR-199, miR-199a-1, miR-200b, miR-201, miR-203, miR-204, miR-206, miR-207, miR-208, miR-210, miR-211, miR-212, miR-213, miR-214, miR-215, miR-216, miR-217, miR-218, miR-222, miR-223, miR-224, miR-291-3p, miR-292, miR-292-3p, miR-293, miR-294, miR-295, miR-296, miR-297, miR-298, miR-299, miR-320, miR-321, miR-322, miR-324, miR-325, miR-326, miR-328, miR-329, miR-330, miR-331, miR-333, miR-335, miR-337, miR-338, miR-340, miR-341, miR-342, miR-344, miR-345, miR-346, miR-350, miR-367, miR-368, miR-369, miR-370, miR-371, miR-373, miR-380-3p, miR-409, miR-410, or miR-412.

Moreover, methods can involve providing synthetic or nonsynthetic miRNA molecules. It is contemplated that in these embodiments, methods may or may not be limited to providing only one or more synthetic miRNA molecules or only on or more nonsynthetic miRNA molecules. Thus, in certain embodiments, methods may involve providing both synthetic and nonsynthetic miRNA molecules. In this situation, a cell or cells are most likely provided a synthetic miRNA molecule corresponding to a particular miRNA and a nonsynthetic miRNA molecule corresponding to a different miRNA. Furthermore, any method articulated a list of miRNAs using Markush group language may be articulated without the Markush group language and a disjunctive article (*i.e.*, or) instead, and vice versa.

In some embodiments, there is a method for reducing or inhibiting cell proliferation in a cell comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves introducing into the cell an effective amount of i) an miRNA inhibitor molecule having a 5′ to 3′ sequence that is at least 90% complementary to the 5′ to 3′ sequence of a mature miRNA selected from the group consisting of: mir-31, mir-92, mir-99a, mir-100, mir-125a, mir-129, mir-130a, mir-150, mir-187, miR-190, miR-191, miR-193, miR 204, mir-210, mir-211, mir-212, mir-213, mir-215, mir-216, mir-217, miR 218, mir-224, mir-292, mir-294, mir-320, mir-324, mir-325, mir-326, mir-330, mir-331, mir-338, mir-341, mir-369, and mir-370; or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence selected from the group consisting of: miR-15a, miR-16, miR 21, miR 24, miR-96, miR-101, miR-105, miR-124, miR-126, miR-142, miR-147, miR-192, miR-194, miR-206, miR-215, or miR-346.

In addition or alternatively, any of the following may be included in the group from which the miRNA inhibitor molecule (i) may be chosen: Let-7a, Let-7b, Let-7c, Let-7d, Let-7g, miR-7, mir-9, miR-10a, miR-10b, miR-18, miR-19a, miR-17-3p, miR-20, miR-23b, mir-25, miR-26a, miR-26a, mir-30e-5p, mir-31, mir-32, mir-92, mir-93, miR-100, miR-125a, miR-125b, mir-127, miR-128, miR-129, mir-130a, mir-135, mir-138, mir-139, miR-140, mir-141, mir-143, mir-145, mir-146, miR-150, mir-154, mir-155, mir-181a, miR-182, mir-186, miR-187, miR-188, mir-190, mir-191, mir-193, mir-196, mir-197, mir-198, mir-199, mir-201, mir-204, mir-216, mir-218, miR-223, mir-293, miR-291-3p, miR-294, miR-295, miR-322, mir-333, mir-335, mir-338, mir-341, mir-350, mir-369, miR-373, mir-410, and mir-412. In addition or alternatively, any of the following may be included in the group from which the miRNA molecule that corresponds to an miRNA sequence (ii) may be chosen: let7a-1, Let-7a, Let-7b, let7b-1, let7c, let7d, Let-7g, mir-9, mir-10a, mir-10b, mir-15a, mir-16, mir-21, mir-23a, mir-23b, mir-24, mir-25, mir-92, mir-95, mir-133a, mir-133a-2, mir-133b, mir-142, mir-152, mir-153, mir-155, mir-181a, mir-182, mir-183, mir-184, mir-186, mir-187, mir-191, mir-193, mir-194, mir-196, mir-199a-1, mir-200b, mir-204, mir-206, mir-211, mir-222, mir-223, mir-298, mir-328, mir-342, mir-371, and mir-412.

In other words, methods involve providing a synthetic miRNA inhibitor having a sequence that is at least 90% complementary to the 5′ to 3′ sequence of a corresponding miRNA that is mir-31, mir-92, mir-99a, mir-100, mir-125a, mir-129, mir-130a, mir-150, mir-187, miR-190, miR-191, miR-193, miR 204, mir-210, mir-211, mir-212, mir-213, mir-215, mir-216, mir-217, miR 218, mir-224, mir-292, mir-294, mir-320, mir-324, mir-325, mir-326, mir-330, mir-331, mir-338, mir-341, mir-369, mir-370, Let-7a, Let-7b, Let-7c, Let-7d, Let-7g, miR-7, mir-9, miR-10a, miR-10b, miR-18, miR-19a, miR-17-3p, miR-20, miR-23b, mir-25, miR-26a, miR-26a, mir-30e-5p, mir-31, mir-32, mir-92, mir-93, miR-100, miR-125a, miR-125b, mir-127, miR-128, miR-129, mir-130a, mir-135, mir-138, mir-139, miR-140, mir-141, mir-143, mir-145, mir-146, miR-150, mir-154, mir-155, mir-181a, miR-182, mir-186, miR-187, miR-188, mir-190, mir-191, mir-193, mir-196, mir-197, mir-198, mir-199, mir-201, mir-204, mir-216, mir-218, miR-223, mir-293, miR-291-3p, miR-294, miR-295, miR-322, mir-333, mir-335, mir-338, mir-341, mir-350, mir-369, miR-373, mir-410, or mir-412. Alternatively or additionally, methods involve providing a synthetic or nonsynthetic miRNA molecule that corresponds to miR-15a, miR-16, miR 21, miR 24, miR-96, miR-101, miR-105, miR-124, miR-126, miR-142, miR-147, miR-192, miR-194, miR-206, miR-215, miR-346, let7a-1, Let-7a, Let-7b, let7b-1, let7c, let7d, Let-7g, mir-9, mir-10a, mir-10b, mir-15a, mir-16, mir-21, mir-23a, mir-23b, mir-24, mir-25, mir-92, mir-95, mir-133a, mir-133a-2, mir-133b, mir-142, mir-152, mir-153, mir-155, mir-181a, mir-182, mir-183, mir-184, mir-186, mir-187, mir-191, mir-193, mir-194, mir-196, mir-199a-1, mir-200b, mir-204, mir-206, mir-211, mir-222, mir-223, mir-298, mir-328, mir-342, mir-371, or mir-412. Methods for reducing or inhibiting cell proliferation can be used as a treatment for diseases and conditions that include, but are not limited to, hyperproliferative diseases, such as cancer.

The present invention also concerns methods for inducing or increasing cell proliferation in a cell comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves introducing into or providing the cell an effective amount of i) an miRNA inhibitor corresponding to let7a-1, Let-7a, Let-7b, let7b-1, let7c, let7d, Let-7g, mir-9, mir-10a, mir-10b, mir-15a, mir-16, mir-21, mir-23a, mir-23b, mir-24, mir-25, mir-92, mir-95, mir-133a, mir-133a-2, mir-133b, mir-142, mir-152, mir-153, mir-155, mir-181a, mir-182, mir-183, mir-184, mir-186, mir-187, mir-191, mir-193, mir-194, mir-196, mir-199a-1, mir-200b, mir-204, mir-206, mir-211, mir-222, mir-223, mir-298, mir-328, mir-342, mir-371, and mir-412; or ii) a miRNA molecule corresponding to Let-7a, Let-7b, Let-7c, Let-7d, Let-7g, miR-7, mir-9, miR-10a, miR-10b, miR-15a, miR-18, miR-19a, miR-17-3p, miR-20, miR-23b, mir-25, miR-26a, miR-26a, mir-30e-5p, mir-31, mir-32, mir-92, mir-93, miR-100, miR-125a, miR-125b, miR-126, mir-127, miR-128, miR-129, mir-130a, mir-135, mir-138, mir-139, miR-140, mir-141, mir-143, mir-145, mir-146, miR-150, mir-154, mir-155, mir-181a, miR-182, mir-186, miR-187, miR-188, mir-190, mir-191, mir-193, mir-194, mir-196, mir-197, mir-198, mir-199, mir-201, mir-204, mir-216, mir-218, miR-223, mir-293, miR-291-3p, miR-294, miR-295, miR-322, mir-333, mir-335, mir-338, mir-341, mir-350, mir-369, miR-373, mir-410, and mir-412. Alternatively or additionally, the group of miRNA inhibitors includes miR-15a, miR-16, miR 21, miR 24, miR-96, miR-101, miR-105, miR-124, miR-126, miR-142, miR-147, miR-192, miR-194, miR-206, miR-215, or miR-346 and the group of miRNAs molecules corresponding to miRNAs includes mir-31, mir-92, mir-99a, mir-100, mir-125a, mir-129, mir-130a, mir-150, mir-187, miR-190, miR-191, miR-193, miR 204, mir-210, mir-211, mir-212, mir-213, mir-215, mir-216, mir-217, miR 218, mir-224, mir-292, mir-294, mir-320, mir-324, mir-325, mir-326, mir-330, mir-331, mir-338, mir-341, mir-369, and mir-370.

Such methods can be used for the treatment of wounds, burns, ischemia, or any other condition, disease, or symptom in which cell proliferation is desirable.

It will be understood in methods of the invention that a cell or other biological matter such as an organism (including patients) can be provided an miRNA or miRNA molecule corresponding to a particular miRNA by administering to the cell or organism a nucleic acid molecule that functions as the corresponding miRNA once inside the cell. The form of the molecule provided to the cell may not be the form that acts an an miRNA once inside the cell. Thus, it is contemplated that in some embodiments, biological matter is provided a synthetic miRNA or a nonsynthetic miRNA, such as one that becomes processed into a mature and active miRNA once it has access to the cell's miRNA processing machinery. In certain embodiments, it is specifically contemplated that the miRNA molecule provided to the biological matter is not a mature miRNA molecule but a nucleic acid molecule that can be processed into the mature miRNA once it is accessible to miRNA processing machinery. The term "nonsynthetic" in the context of miRNA means that the miRNA is not "synthetic," as defined herein. Furthermore, it is contemplated that in embodiments of the invention that concern the use of synthetic miRNAs, the use of corresponding nonsynthetic miRNAs is also considered an aspect of the invention, and vice versa.

In other embodiments, the methods involve reducing cell viability comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves introducing into one or more cells an effective amount of i) an miRNA inhibitor corresponding to miR-107, miR-133, miR-137, miR-152, miR-155, miR-181a, miR-191, miR-203, or miR-215; or ii) an miRNA molecule corresponding to let-7a, let-7b, mir-1, mir-7, miR-10b, miR-17-3p, miR-19a, mir-23, mir-24, mir-27a, miR-29a, miR-30a-3p, mir-31, mir-32, miR-34a, miR-101, miR-107, miR-108, miR-122, mir-124, miR-133a, miR-134, miR-135, miR-139, mir-140, miR-141, miR-145, mir-150, mir-192, mir-193, mir-195, mir-206, mir-208, mir-210, mir-210, mir-292-3p, mir-293, mir-297, mir-299, mir-329, mir-337, mir-337, mir-345, mir-346, and mir-409. Alternatively or additionally, the group of miRNA inhibitors (group i) includes let-7a, let-7b, let-7c, let-7d, let-7g, miR-10a, miR-10b, miR-15a, miR-17-3p, miR-18, miR-19a, miR-20, mir-23a, mir-23b, mir-24, miR-25, miR-26a, mir-32, miR-107, miR-125a, miR-126, mir-128, miR-129, miR-133, miR-137, mir-139, miR-143, miR-152, miR-155, miR-181a, miR-182, miR-191, miR-203, miR-215, and mir-331

Other aspects of the invention include a method for increasing cell viability comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves introducing into one or more cells an effective amount of i) an miRNA inhibitor corresponding to miR-7, miR-19a, miR-23, miR-24, miR-27a, miR-31, miR-32, miR-134, miR-140, miR-150, miR-192, or miR-193; or ii) an miRNA molecule corresponding to let-7a, let-7b, let-7c, let-7d, let-7g, miR-10a, miR-10b, miR-15a, miR-17-3p, miR-18, miR-19a, miR-20, mir-23a, mir-23b, mir-24, miR-25, miR-26a, mir-32, miR-107, miR-125a, miR-126, mir-128, miR-129, miR-133, miR-137, mir-139, miR-143, miR-152, miR-155, miR-181a, miR-182, miR-191, miR-203, miR-215, and mir-331. Alternatively or additionally, the group of miRNA inhibitors (group i) includes let-7a, let-7b, mir-1, mir-7, miR-10b, miR-17-3p, miR-19a, mir-23, mir-24, mir-27a, miR-29a, miR-30a-3p, mir-31, mir-32, miR-34a, miR-101, miR-107, miR-108, miR-122, mir-124, miR-133a, miR-134, miR-135, miR-139, mir-140, miR-141, miR-145, mir-150, mir-192, mir-193, mir-195, mir-206, mir-208, mir-210, mir-210, mir-292-3p, mir-293, mir-297, mir-299, mir-329, mir-337, mir-337, mir-345, mir-346, or mir-409, and the group of miRNAs molecules corresponding to miRNAs (group ii) includes .The present invention also concerns a method for inducing apoptosis in a cell comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves introducing into the cell an effective amount of i) an miRNA inhibitor corresponding to miR-31 or miR-214; or ii) an miRNA molecule corresponding to let-7b, let-7g, mir-1, mir-1d, mir-7, mir-10a, miR-10b, miR-17-3p, miR-19a, miR-28, miR-28, miR-28, miR-29a, miR-32, miR-34a, miR-122, mir-148, mir-149, mir-154, mir-184, mir-186, mir-188, mir-192, mir-195, mir-196, mir-199a, mir-204, mir-208,mir-210, mir-211, mir-212, mir-214, mir-215, mir-216, mir-217, mir-218, mir-293, mir-296, mir-299, mir-321, mir-328, or mir-344. Alternatively or additionally, the group of miRNA inhibitors (group i) includes Let-7b, mir-21, mir-23b, mir-25, miR-26a, mir-28, mir-29a, mir-31, miR-32, mir-30a-3p, mir-34a, mir-96, miR-98, mir-100, mir-101, mir-105, mir-108 , miR-125b, miR-126, mir-126, mir-128, mir-137, miR-143, miR-155, mir-207, mir-214, mir-216, mir-223, mir-292-3p, mir-328, mir-335, mir-340, mir-341, mir-367, mir-368, mir-380-3p, and mir-410 .

Methods for inducing apoptosis have a number of therapeutic applications including, but not limited to, the treatment of cancer.

Other embodiments of the invention involve a method for inhibiting apoptosis in a cell comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves introducing into the cell an effective amount of i) an miRNA inhibitor corresponding to miR-7, miR-1-2, miR-148, miR-195, miR-196, miR-199a, miR-204, miR-210, miR-211, miR-212, miR-215, miR-216, miR-218, miR-296, or miR-321; or ii) an miRNA molecule corresponding to Let-7b, mir-21, mir-23b, mir-25, miR-26a, mir-28, mir-29a, mir-31, miR-32, mir-30a-3p, mir-34a, mir-96, miR-98, mir-100, mir-101, mir-105, mir-108 , miR-125b, miR-126, mir-126, mir-128, mir-137, miR-143, miR-155, mir-207, mir-214, mir-216, mir-223, mir-292-3p, mir-328, mir-335, mir-340, mir-341, mir-367, mir-368, mir-380-3p, or mir-410. Alternatively or additionally, the group of miRNA inhibitors (group i) includes let-7b, let-7g, mir-1, mir-1d, mir-7, mir-10a, miR-10b, miR-17-3p, miR-19a, miR-28, miR-28, miR-28, miR-29a, miR-32, miR-34a, miR-122, mir-148, mir-149, mir-154, mir-184, mir-186, mir-188, mir-192, mir-195, mir-196, mir-199a, mir-204, mir-208,mir-210, mir-211, mir-212, mir-214, mir-215, mir-216, mir-217, mir-218, mir-293, mir-296, mir-299, mir-321, mir-328, or mir-344.

The present invention also concerns using miRNA compositions to treat diseases or conditions or to prepare therapeutics for the treatment of diseases or conditions. In some embodiments, the invention involves one or more human miRNA selected from the group consisting of let-7, miR-10a, miR-15a, miR-16, miR-17, miR-21, miR-22, miR-23, miR-24, miR-26a, miR-29b, miR-30a, miR-96, miR-101, miR-105, miR-106, miR-124, miR-125a, miR-126, miR-130, miR130a, miR-133, miR-142, miR-143, miR-144, miR-145, miR-147, miR-181a, miR-182, miR-183, miR-188, miR-189, miR-192, miR-194, miR-195, miR-199a, miR-200b, miR-201, miR-205, miR-219, 206, miR-215, miR-219, miR-223, miR-224, miR-321, miR-328, miR-331, miR-342. and miR-219,346. It is contemplated that 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 more miRNA (or any range derivable therein) may be used for these embodiments. In certain embodiments, methods involve one or more miRNA inhibitors and/or an miRNA molecules corresponding to any of these miRNAs, particularly for the treatment or prevention of cancer. Cancer includes, but is not limited to, malignant cancers, tumors, metastatic cancers, unresectable cancers, chemo- and/or radiation-resistant cancers, and terminal cancers.

In some embodiments of the invention, methods involve one or more miRNA inhibitors and/or an miRNA molecules corresponding to miR-17, miR-21, miR-126, miR-143. miR-145, miR-188, miR-200b, miR-219, or miR-331. In certain embodiments, methods involve one or more of 1) an inhibitor of miR-17, miR-21, miR-182, miR-183, miR-200b, miR-205, miR-223, and/or miR-224; and/or 2) an miRNA corresponding to let-7, miR-10a, miR-16, miR-22, miR-23, miR-24, miR-26a, miR-29b, miR-30a, miR-106, miR-125a, miR-126, miR-130, miR-133, miR-143, miR-144, miR-145, miR-181a, miR-188, miR-219, miR-192, miR-194, miR-195, miR-199a, mmu-miR-201, miR-215, miR-321, miR-328, miR-331, and/or miR-342. Such methods can be used, in some embodiments to treat cancer, including specific cancers. Additionally, an miRNA corresponding to one or more of miR-15a, miR-16, miR-96, miR-101, miR-105, miR-124, miR-126, miR-142, miR-147, miR-192, miR-194, miR-206, miR-215, or miR-346 may be used to treat cancer or inhibit cell proliferation. It is contemplated that these miRNAs may be used regardless of the source of the cell in which proliferation is undesirable.

It will be understood that shorthand notations are employed such that a generic description of an miRNA refers to any of its gene family members (distinguished by a number), unless otherwise indicated. It is understood by those of skill in the art that a "gene family" refers to a group of genes having the same miRNA coding sequence. Typically, members of a gene family are identified by a number following the initial designation. For example, miR-16-1 and miR-16-2 are members of the miR-16 gene family and "mir-7" refers to miR-7-1, miR-7-2 and miR-7-3. Moreover, unless otherwise indicated, a shorthand notation refers to related miRNAs (distinguished by a letter). Thus, "let-7," for example, refers to let-7a-1, let7-a-2, let-7b, let-7c, let-7d, let-7e, let-7f-1, and let-7f-2." Exceptions to this shorthand notations will be otherwise identified.

The present invention concerns treating breast cancer or decreasing cell proliferation of breast cancer cells by introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing an effective amount of at least 1) one or more miRNA inhibitors corresponding to miR-21, miR-15a, miR-16, miR-24, and/or miR-25, and/or 2) one or more miRNAs corresponding to miR-99, miR-100, miR-205, miR-197, miR-126, miR-143, miR-145 and/or miR-321. Alternatively or additionally, the miRNAs molecules corresponding to miRNAs (group ii) can include mir-27a, mir-92, mir-96, mir-98, mir-99a, mir-101, mir-105, mir-124, mir-126, mir-129, mir-132, mir-

142, mir-147, mir-192, mir-201, mir-206, mir-208, mir-210, mir-211, mir-214, mir-215, mir-219, mir-220, mir-221, mir-223, mir-297, mir-329, mir-331, mir-345, mir-346, mir-409, or mir-411.

It will be understand that the term “providing” an agent is used to include “administering” the agent to a patient.

The present invention also relates to treating colon cancer by introducing into or providing to a colon cancer cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing 1) one or more miRNA inhibitors corresponding to miR-21, miR-106, miR-200b, miR-223, miR-224, miR-31, and/or miR-17; and/or 2) one or more miRNAs corresponding to miR-145, miR-143, miR-133, miR-342, miR-125a, miR-195, miR-30a, miR-10a, miR-130, miR-192, miR-194, miR-215, miR-144, miR-23, miR-26a, miR-126, miR-199a, miR-188, miR-331, and/or miR-21.

Moreover, methods for treating thyroid cancer involve introducing into or providing to a thyroid cancer cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to the patient 1) one or more miRNA inhibitors corresponding to miR-21 miR-125, miR-24, miR-200b, miR-29b, miR-221, miR-222, miR-224, miR-10a, and/or miR-183; and/or 2) one or more miRNAs corresponding to miR-145, miR-22, miR-331, miR-126, miR-30a, miR-199a, miR-223, and/or miR-321.

The treatment of lung cancer is also contemplated as part of the invention. Methods involve introducing into or providing to a lung cancer cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to the patient 1) one or more miRNA inhibitors corresponding to miR-223, miR-106, miR-21, miR-200b, miR-321, miR-182, miR-183, miR-17, and/or miR-205; and/or 2) one or more miRNAs corresponding to miR-130a, miR-145, miR-126, miR-331, miR-342, miR-143, Let-7, miR-30a, miR-16, miR-26a, miR-125a, miR-29b, miR-24, miR-328, miR-195, miR-22, miR-181a, miR-331, and/or miR-321. Alternatively or additionally, the group of miRNA inhibitors (group 1) includes mir-30e-5p, mir-25, mir-32, mir-92, mir-130a, mir-135, mir-145, mir-216, mir-293, mir-294, mir-333, mir-335, mir-338, mir-341, mir-350, mir-369, or mir-412, and the group of miRNAs molecules corresponding to miRNAs (group 2) includes ambi-mir7100, Let-7b, Let-7d, Let-7g, mir-7, mir-15a, mir-16, mir-22, mir-28, mir-29a, mir-34a, mir-96, mir-101, mir-105, mir-108, mir-122, mir-124, mir-125a, mir-125b, mir-126, mir-128, mir-129, mir-132, mir-133A, mir-136, mir-137, mir-141, mir-142, mir-147, mir-149, mir-151, mir-152, mir-182, mir-183, mir-186, mir-188, mir-192, mir-193, mir-195, mir-223, mir-292-3p, mir-337, mir-337, mir-344, mir-345, mir-346, mir-377, or mir-526b*.

The present invention concerns treating cervical cancer or decreasing cell proliferation of cervical cancer cells by providing an effective amount of at least 1) one or more miRNA inhibitors corresponding to Let-7a, Let-7b, Let-7c, Let-7d, Let-7g, mir-9, mir-145, mir-155, mir-181a, mir-186, mir-190, mir-191, or mir-199, and/or 2) one or more miRNAs corresponding to mir-1, mir-34a, mir-101, mir-124, mir-192, mir-193, mir-195, mir-201, mir-206, mir-208, mir-210, mir-215, mir-292-3p , mir-293, mir-297, mir-299, mir-337, mir-339, mir-340, mir-344, mir-345, mir-367, or mir-409.

The present invention concerns treating prostate cancer or decreasing cell proliferation of prostate cancer cells by introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing an effective amount of at least 1) one or more miRNA inhibitors corresponding to Let-7a, Let-7b, mir-93, mir-127, mir-154, mir-181a, mir-194, mir-198, mir-199, mir-201, or mir-369, and/or 2) one or more miRNAs corresponding to mir-15a, mir-16, mir-27a, mir-28, mir-30a-3p, mir-34a, mir-101, mir-103, mir-105, mir-107, mir-124, mir-126, mir-128, mir-129, mir-132, mir-135, mir-137, mir-141, mir-142, mir-147, or mir-297.

The present invention concerns treating skin cancer or decreasing cell proliferation of skin cancer cells by introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing an effective amount of at least 1) one or more miRNA inhibitors corresponding to miR-26a, miR-125a, miR-128, mir-138, mir-139, mir-141, mir-143, miR-145, mir-146, miR-150, miR-187, mir-188, mir-190, mir-196, mir-197, mir-198, mir-199, miR-201, mir-204, mir-216, miR-223, miR-291-3p, miR-294, miR-295, miR-322, miR-373, mir-410, or mir-412, and/or 2) one or more miRNAs corresponding to let 7a, mir-1, mir-7, mir-15a, mir-16, mir-20, mir-26a, mir-28, mir-34a, mir-96, mir-101, mir-105, miR-105, mir-124, mir-126, mir-128, mir-132, mir-133A, mir-136, mir-137, mir-141, mir-142, mir-144, miR-147, mir-154, mir-181a, mir-192, mir-193, miR-195, mir-201, mir-206, mir-206, mir-215, mir-221, mir-223, mir-291, miR-297, mir-302, miR-324-3p, mir-329, mir-330, miR-337, mir-346, mir-346, mir-373, mu-mir-376b, mir-380-3p, or mir-411.The present invention concerns treating leukemia or decreasing cell proliferation of cancerous T cells by introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing an effective amount of at least 1) one or more miRNA inhibitors corresponding to miR-15a, miR-23b, miR-25, miR-26a, miR-100, miR-125b, miR-126, miR-129, miR-140, miR-143, or miR-155, and/or 2) one or more miRNAs corresponding to let-7a, let-7b, miR-10b, miR-17-3p, miR-29a, miR-30a-3p, miR-34a, miR-101, miR-122, or miR-133a. Alternatively or additionally, the group of miRNA inhibitors (group 1) includes let-7a, let-7b, let-7c, let-7d, let-7g, miR-7, miR-10a, miR-10b, miR-15a, miR-17-3p, miR-18, miR-19a, miR-20, miR-125a, miR-126, or miR-182, and the group of miRNAs molecules corresponding to miRNAs (group 2) includes miR-107, miR-134, miR-135, miR-139, miR-141, or miR-145. Moreover, such methods can extend to T-cells generally.

In addition to any miRNAs disclosed herein in the context of decreasing cell proliferation, embodiments of the invention include methods for decreasing cell proliferation comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing or introducing an effective amount of at least 1) one or more miRNA inhibitors corresponding to Let-7a, Let-7b, Let-7c, Let-7d, Let-7g, miR-7, mir-9, miR-10a, miR-10b, miR-15a, miR-18, miR-19a, miR-17-3p, miR-20, miR-23b, mir-25, miR-25, miR-26a, miR-26a, mir-30e-5p, mir-32, mir-92, mir-93, miR-100, miR-125a, miR-125b, miR-126, mir-127, miR-128, miR-129, mir-130a, mir-135, mir-

138, mir-139, miR-140, mir-141, mir-143, mir-145, mir-146, miR-150, mir-154, mir-155, mir-181a, miR-182, mir-186, miR-187, miR-188, mir-190, mir-191, mir-194, mir-196, mir-197, mir-198, mir-199, mir-201, mir-204, mir-216, miR-223, mir-293, miR-291-3p, miR-294, miR-295, miR-322, mir-333, mir-335, mir-338, mir-341, mir-350, mir-369, miR-373, mir-410, or mir-412, and/or 2) one or more miRNAs corresponding to ambi-mir7100, let 7a, let-7b, let-7d, let-7g, mir-1, mir-7, miR-10b, mir-15a, mir-16, miR-17-3p, mir-20, mir-22, mir-26a, mir-27a, mir-28, mir-28, miR-29a, mir-30a-3p, mir-34a, mir-92, mir-96, mir-98, mir-99a, mir-101, mir-103, mir-105, mir-107, mir-108, mir-122, mir-124, mir-125a, mir-125b, mir-126, mir-128, mir-129, mir-132, miR-133a, miR-134, mir-135, mir-136, mir-137, miR-139, mir-141, mir-142, mir-144, miR-145, mir-147, mir-149, mir-151, mir-152, mir-154, mir-181a, mir-182, mir-183, mir-186, mir-188, mir-192, mir-193, mir-195, mir-195, mir-201, mir-206, mir-208, mir-210, mir-211, mir-214, mir-215, mir-219, mir-220, mir-221, mir-223, mir-291, mir-292-3p, mir-293, mir-297, mir-299, mir-302, miR-324-3p, mir-329, mir-330, mir-331, mir-337, mir-339, mir-340, mir-344, mir-345, mir-346, mir-367, mir-373, miR-376b, mir-377, mir-380-3p, mir-409, mir-411, or mir-526b*. It is particularly contemplated that such methods may be employed in the context of treating cancer or another disease or condition in which cell proliferation plays a role, such as hyproliferative diseases and conditions.

The present invention also concerns embodiments methods for increasing cell proliferation comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing or introducing an effective amount of at least 1) one or more miRNA inhibitors corresponding to ambi-mir7100, let 7a, let-7b, let-7d, let-7g, mir-1, mir-7, miR-10b, mir-15a, mir-16, miR-17-3p, mir-20, mir-22, mir-26a, mir-27a, mir-28, mir-28, miR-29a, mir-30a-3p, mir-34a, mir-92, mir-96, mir-98, mir-99a, mir-101, mir-103, mir-105, mir-107, mir-108, mir-122, mir-124, mir-125a, mir-125b, mir-126, mir-128, mir-129, mir-132, miR-133a, miR-134, mir-135, mir-136, mir-137, miR-139, mir-141, mir-142, mir-144, miR-145, mir-147, mir-149, mir-151, mir-152, mir-154, mir-181a, mir-182, mir-183, mir-186, mir-188, mir-192, mir-193, mir-195, mir-195, mir-201, mir-206, mir-208, mir-210, mir-211, mir-214, mir-215, mir-219, mir-220, mir-221, mir-223, mir-291, mir-292-3p, mir-293, mir-297, mir-299, mir-302, miR-324-3p, mir-329, mir-330, mir-331, mir-337, mir-339, mir-340, mir-344, mir-345, mir-346, mir-367, mir-373, miR-376b, mir-377, mir-380-3p, mir-409, mir-411, or mir-526b*, and/or 2) one or more miRNAs corresponding to Let-7a, Let-7b, Let-7c, Let-7d, Let-7g, miR-7, mir-9, miR-10a, miR-10b, miR-15a, miR-18, miR-19a, miR-17-3p, miR-20, miR-23b, mir-25, miR-25, miR-26a, miR-26a, mir-30e-5p, mir-32, mir-92, mir-93, miR-100, miR-125a, miR-125b, miR-126, mir-127, miR-128, miR-129, mir-130a, mir-135, mir-138, mir-139, miR-140, mir-141, mir-143, mir-145, mir-146, miR-150, mir-154, mir-155, mir-181a, miR-182, mir-186, miR-187, miR-188, mir-190, mir-191, mir-194, mir-196, mir-197, mir-198, mir-199, mir-201, mir-204, mir-216, miR-223, mir-293, miR-291-3p, miR-294, miR-295, miR-322, mir-333, mir-335, mir-338, mir-341, mir-350, mir-369, miR-373, mir-410, or mir-412. While not limited to such an embodiment, one use for such a method is to increase or induce proliferation of normal cells or other desirable cells in the context of pretreatment or therapy.

Other aspects of the invention include the treatment of systemic lupus erythrematosus (SLE). In certain embodiments, methods concern introducing into or providing to a patient an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to a patient with SLE or suspect of having SLE 1) one or more miRNA inhibitors corresponding to miR-21, miR-223, and/or mir-342 expression; and/or 2) one or more miRNAs corresponding to miR-95, miR-105, miR-137, miR-186, miR-188, miR-199, miR-211, miR-215, mu-miR-290, miR-301, and/or miR-331.

Treatment or prevention of prion diseases is included in methods of the invention. In some cases, method include introducing into or providing to a patient with a prion disease an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to a patient 1) one or more miRNA inhibitors corresponding miR-7, miR-9, miR-16, miR-24, miR-26A, miR-27A, and/or miR-130A; and/or 2) one or more miRNAs corresponding to miR-95 and/or miR-135A. The patient may be one diagnosed with a prion disease, one at risk for a prion disease, or one suspected of having a prion disease. It is specifically contemplated that in some embodiments of the invention, a nucleic acid molecule corresponding to an miRNA is double stranded, wherein both strands have the sequence of the mature miRNA it corresponds to. Such a molecule may be designated with an "as" suffix in embodiments of the invention. For example, a nucleic acid molecule called miR-9-as was used in some experiments described herein. It is contemplated that in some embodiments, a nucleic acid molecule is an miRNA-as molecule.

The present invention also concerns patients diagnosed as having ischemia, those at risk for ischemia, those suspected of having ischemia, or patients with symptoms of ischemia. Methods involve introducing into or providing to a patient an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to a patient 1) one or more miRNA inhibitors corresponding to miR-28, miR-30A, miR-31, miR-138, miR-139, miR-140, miR-291 and/or mmu-miR-298; and/or 2) one or more miRNAs corresponding to Let-7f-2 and/or miR-16.

In certain experiments, a synthetic miRNA in which both the sense and antisense strand are derived from a single precursor miRNA is used in methods and compositions of the invention. These are frequently designated with a "P" suffix in which "5P" indicates that the mature miRNA derives from the 5' end of the precursor and a corresponding "3P" indicates that it dferives from the 3' end of the precursor, as described on the world wide web at sanger.ac.uk/cgi-bin/rfam/mirna. Moreover, in some embodiments, an miRNA that does not correspond to a known human miRNA was evaluated. It is contemplated that these non-human miRNAs may be used in embodiments of the invention or that there may exist a human miRNA that is homologous to the non-human miRNA.

The present invention in some embodiments concerns methods for reducing cell viability comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is let-7a, let-7b, miR-1, miR-10b, miR-17, miR-19a, miR-20, miR-28, miR-29a, miR-30a, miR-32, miR-34a, miR-96, miR-101, miR-122, miR-124, miR-132, miR-133a, miR-134, miR-139, miR-140, miR-144, miR-145, miR-147, miR-155, miR-182, miR-183, miR-184, miR-186, miR-190, miR-193, miR-197, miR-206, miR-208, miR-210, miR-216, miR-217, miR-224, mu-miR-292, mu-miR-293, mu-miR-298, miR-299, miR-301, mu- miR-329, miR-337, mu-miR-344, miR-345, miR-346, miR-369, mu-miR-380, or mu-miR-409; or 2) at least one miRNA inhibitor corresponding to let-7a, let-7b, let-7c, miR-9, miR-10a, miR-10b, miR-15a, miR-17, miR-18, miR-20, mir-23b, miR-25, miR-26a, miR-98, miR-100, miR-125a, miR-125b, miR-126, miR-129, miR-140, miR-141, miR-143, miR-155, or miR-181-a. The term “reducing cell viability” means reducing the number of live cells.

Methods concerning cell viability and cell proliferation may generally be used for therapeutics, diagnostics, creating cell lines with interesting research properties, and inducing differentiation. miRNAs that selectively reduce the proliferation of cancer cells may be employed as therapeutics since they can be delivered to cancer and non-cancer cells alike but will only affect the growth of the cancerous cells. In addition, methods may be used to halt or prevent metastasis or reduce the number of metastases.

It is contemplated in some embodiments that the cell in which the effect is desired (referred to as a “targeted cell”), such as a reduction in cell viability, may be a cell that is diseased or involved in maintaining, promoting, or causing a disease or condition. In certain embodiments, the cell is a cancer cell, while in other embodiments, it is contemplated to be a healthy (non-diseased) cell. In certain embodiments, a targeted cell is in an organism.

Moreover, it is particularly contemplated that a nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell is a synthetic miRNA in some embodiments of the invention.

In other embodiments, the present invention involves methods for increasing cell viability comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is let-7a, let-7b, let-7c, miR-9, miR-10a, miR-10b, miR-15a, miR-17, miR-18, miR-20, mir-23b, miR-25, miR-26a, miR-98, miR-100, miR-125a, miR-125b, miR-126, miR-129, miR-140, miR-141, miR-143, miR-155, or miR-181-a; or 2) at least one miRNA inhibitor corresponding to let-7a, let-7b, miR-1, miR-10b, miR-17, miR-19a, miR-20, miR-28, miR-29a, miR-30a, miR-32, miR-34a, miR-96, miR-101, miR-122, miR-124, miR-132, miR-133a, miR-134, miR-139, miR-140, miR-144, miR-145, miR-147, miR-155, miR-182, miR-183, miR-184, miR-186, miR-190, miR-193, miR-197, miR-206, miR-208, miR-210, miR-216, miR-217, miR-224, mu-miR-292, mu-miR-293, mu-miR-298, miR-299, miR-301, mu- miR-329, miR-337, mu-miR-344, miR-345, miR-346, miR-369, mu-miR-380, or mu-miR-409. The term “increasing cell viability” means that cell death is inhibited. In particular embodiments, a cancer cell, such as a leukemia cell, is provided with an effective amount of a nucleic acid capable of being processed into a mature let-7a, let-7b, or miR-10b molecule.

Methods of the invention also relate to inhibiting cellular proliferation comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is let-7a, let-7b, let-7c, let-7d, let-7g, miR-1, miR-7, miR-15a, miR-16, miR-19a, miR-22, miR-28, miR-29a, miR-34a, miR-92, miR-96, miR-98, miR-101, miR-122, miR-124, miR-126, miR-129, miR-133b, miR-137, miR-147, miR-192, miR-193, miR-195, miR-205, miR-206, miR-208, miR-210, mu-miR-292, mu-miR-297, miR-299, miR-337, mu-miR-344, miR-345, or miR-346; or 2) at least one miRNA inhibitor corresponding to miR-25, miR-27a, miR-31, miR-32, miR-92, miR-139, miR-145, miR-198, miR-212, mu-miR-290, mu-miR-294, miR-323, miR-324, miR-325, miR-331, miR-335, mu-miR-351, miR-369, miR-370, or miR-373.

In some embodiments there are methods of increasing cellular proliferation comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is miR-25, miR-27a, miR-31, miR-32, miR-92, miR-139, miR-145, miR-198, miR-212, mu-miR-290, mu-miR-294, miR-323, miR-324, miR-325, miR-331, miR-335, mu-miR-351, miR-369, miR-370, or miR-373; or 2) at least one miRNA inhibitor corresponding to let-7a, let-7b, let-7c, let-7d, let-7g, miR-1, miR-7, miR-15a, miR-16, miR-19a, miR-22, miR-28, miR-29a, miR-34a, miR-92, miR-96, miR-98, miR-101, miR-122, miR-124, miR-126, miR-129, miR-133b, miR-137, miR-147, miR-192, miR-193, miR-195, miR-205, miR-206, miR-208, miR-210, mu-miR-292, mu-miR-297, miR-299, miR-337, mu-miR-344, miR-345, or miR-346.

The present invention also covers methods of inhibiting ERK activation introducing into or providing to a cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves comprising providing to or introducing into cells an effective amount of one or more miRNA inhibitor corresponding to let-7a, mir-294, mir-295, miR-19a, miR-25, miR-96, miR-125a, miR-134, miR-148, miR-152, miR-206, miR-207, miR-210, miR-212, miR-216, miR-217, miR-218, miR-223, mu-miR-294, mu-miR-295, miR-301, miR-328, mu-miR-329, miR-339, miR-370, or miR-372.

In certain embodiments, it also covers methods of activating ERK by introducing into or providing to a cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing or introducing into cells an effective amount of one or more nucleic acids capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is miR-19a, miR-25, miR-96, miR-125a, miR-134, miR-148, miR-152, miR-206, miR-207, miR-210, miR-212, miR-216, miR-217, miR-218, miR-223, mu-miR-294, mu-miR-295, miR-301, miR-328, mu-miR-329, miR-339, miR-370, or miR-372. Alternatively or in addition to the mature miRNA is let-7, miR-19a, miR-25, miR-96, miR-125a, miR-134, miR-148, miR-152, miR-206, miR-207, miR-210, miR-212, miR-216, miR-217, miR-218, miR-223, mu-miR-294, mu-miR-295, miR-301, miR-328, mu-miR-329, miR-339, miR-370, or miR-372.

In other embodiments of the invention, there are methods of increasing the percentage of apoptotic cells in a population comprising introducing into or providing to the cells an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) one or more nucleic acid molecules capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is let-7d, miR-22, miR-23a, miR-23b, miR-24, miR-27a, miR-31, miR-128, miR-181a, miR-196, miR-198, miR-199, miR-214, miR-217, mu-miR-290, mu-miR-293, miR-324, miR-338, or mu-miR-412; or 2) an miRNA inhibitor corresponding to miR-34a, miR-96, miR-101, miR-105, miR-126, miR-137, or mu-miR-292. It is specifically contemplated that the population of cells may be diseased or related to a disease or condition.

In further embodiments of the invention, there are methods of decreasing the percentage of apoptotic cells in a population comprising introducing into or providing to the cells an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is miR-34a, miR-96, miR-101, miR-105, miR-126, miR-137, or mu-miR-292; or 2) at least one miRNA inhibitor corresponding to let-7d, miR-22, miR-23a, miR-23b, miR-24, miR-27a, miR-31, miR-128, miR-181a, miR-196, miR-198, miR-199, miR-214, miR-217, mu-miR-290, mu-miR-293, miR-324, miR-338, or mu-miR-412. It is specifically contemplated that the population of cells may be involved in diseases or conditions involving atrophy or the decrease in the number of healthy cells as a result of apoptosis. One or more of the miRNAs that induce apoptosis may be introduced into abnormal cells like cancer cells to induce cell death, providing a therapeutic response. This could be especially beneficial if the apoptosis-inducing synthetic miRNAs were injected directly into tumor tissues or otherwise delivered with high efficiency to primary or metastatic cancer cells. These same miRNAs may be co-delivered with other therapeutic agents like chemotherapies to supplement their activities and evoke a therapeutic response. Alternatively, the miRNAs that reduce apoptosis may be introduced into normal cells at the same time that a chemotherapeutic reagent that induces apoptosis is introduced, providing some level of protection to the normal cells while the cancer cells are induced to undergo cell death. The miRNAs may also be used as targets for diagnostic assays or to differentiate cells or to create cell lines with interesting research properties.

Methods of the invention include methods for inhibiting or preventing hTert activity in a cell comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into the cell an effective amount of at least one i) miRNA inhibitor corresponding to miR-15a, miR-16, miR-21, mir-24, miR-26a, miR-92, miR-105, miR-125a, miR-125b, miR-128, mir-147, miR-195, miR-207, miR-224, miR-295, mir-301, miR-337, mir-368, or mir-371 or ii) a nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is miR-26a, miR-147, mir-195, and mir-368. It is specifically contemplated that it is desirable to inhibit hTert activity in cancer cells or in a patient at risk for or suspected of having cancer. Methods of the invention include methods for inducing hTert activity in a cell comprising providing to or introducing into the cell an effective amount of at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is miR-15a, miR-16, miR-21, mir-24, miR-26a, miR-92, miR-105, miR-125a, miR-125b, miR-128, mir-147, miR-195, miR-207, miR-224, miR-295, mir-301, miR-337, mir-368, or mir-371. Alternatively or additionally, hTert activity may be induced in a cell comprising providing to or introducing into the cell an miRNA inhibitor corresponding to miR-26a, miR-147, mir-195, or mir-368.

In other embodiments of the invention there are methods for identifying an miRNA that inhibits an hTert activating gene product comprising: a) introducing into a cell a candidate miRNA into a cell; and, b) assaying the level of hTert expression or hTert activity in the cell, wherein a reduction in hTert expression or activity compared to a cell lacking the miRNA identifies the miRNA as a potential inhibitor of an hTert activating gene product. In particular embodiments, the sequence of the candidate miRNA was previously evaluated for an ability to inhibit an hTert activating gene product. Computer programs and algorithms may be employed to assess whether a particular miRNA sequence can target a particular cellular gene. In certain embodiments, thTert activating gene product is selected from the group consisting of ACOX1, AKT1, APAF1, COX-5B, COX6, COX7B, CPOX, DUOX2, GPX1, GPX2, GPX4, LPO, MAPK1, MAPK4, MTCO1, NOX3, NOX5, PAOX, PPOX, PRKCA, PRKCD, and TNFRSF6. These methods may be used for combating telomerase activity and cancer progression.The invention also includes methods for inhibiting stimulation of Stat3 in a cell comprising introducing into or providing to the cell an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to the cell an effective amount of an miRNA selected from the group consisting of mir-93, mir-100, mir-134, mir-99a, mir-103, mir-128, mir-129, mir-181b, mir-193, mir-197, mir-212, mir-218, mir-219, mir-302, mir-323, mir-324-3p, mir-325, mir-330, mir-331, mir-340, mmu-mir-350, mir-425, mir-491, mir-518f, mir-520a*. Such methods can be used for treating diseases and conditions characterized by inflammation. These include, but are not limited to, tissue destruction, organ failure or inflammatory diseases such as Rheumatoid arthritis, Psoriasis, Asthma, Inflammatory bowel disease (Crohn's disease and related conditions), Multiple Sclerosis, obstructive pulmonary disease (COPD), Allergic rhinitis (hay fever), and Cardiovascular disease. Additionally, such methods may be used for therapeutics, diagnostics, prognostics, creating cell lines with interesting research properties, and inducing differentiation.

The present invention also concerns methods of influencing the cell cycle of a cell or population of cells. It is contemplated that methods can involve relatively increasing the number of cells in a particular phase of the cell cycle, such as S, G1, G2/M, or when the number of chromosomes is greater than 2N. Alternatively, it can involve inducing DNA synthesis in a cell. One or more of the miRNAs involved in the cell cycle can be used to modulate a cell, particularly a cancer cell, to achieve a therapeutic benefit for a patient with such cells. Such methods may be used, for example, to enhance the efficacy of a therapeutic agent or they may be employed in the context of research, for instance, to synchronize cells so as to generate a more homogeneous population of cells. Moreover, these miRNAs may regulate genes that are involved in controlling cell cycle progression. Mis-expression of one or more of these miRNAs may profoundly affect the cells in which they reside, leading potentially toward cancer or other diseases associated with altered cell cycle regulation. In addition to using these miRNAs as diagnostic analytes, they might also provide targets for treating disease. For instance, a cancer cell that has bypassed a critical cell cycle signal by having a cell cycle-specific miRNA might be returned to normalcy by introducing the miRNA.

Methods of promoting cells to be in S phase can be achieved by introducing into or providing to the cells an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is let-7a, mir-15a, mir-16, mir-20, mir-26a, mir-191, mir-197, mir-205, mir-220, mir-224, mir-290, mir-291, mir-294, mir-295, mir-302, mir-345, mir-372, or mir-411; or 2) at least one miRNA inhibitor corresponding to mir-108, mir-122, mir-128, mir-129, mir-137, mir-142, mir-146, mir-147, mir-186, mir-187, mir-195, mir-297, mir-324-3p, mir-337, or mir-376b.

The invention also includes methods of inhibiting cells to be in S phase by introducing into or providing to the cells an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is mir-108, mir-122, mir-128, mir-129, mir-137, mir-142, mir-146, mir-147, mir-186, mir-187, mir-195, mir-297, mir-324-3p, mir-337, or mir-376b; or 2) at least one miRNA inhibitor corresponding to let-7a, mir-15a, mir-16, mir-20, mir-26a, mir-191, mir-197, mir-205, mir-220, mir-224, mir-290, mir-291, mir-294, mir-295, mir-302, mir-345, mir-372, or mir-411.

Methods of promoting cells to be in G1 phase can be achieved by introducing into or providing to the cells an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is mir-108, mir-122, mir-124, mir-125a, mir-126, mir-128, mir-129, mir-137, mir-142, mir-146, mir-147, mir-195, mir-201, mir-297, mir-320, mir-325, mir-324-3p, mir-337, mir-371, mir-376b, or mir-409; or 2) at least one miRNA inhibitor corresponding to Let-7a, mir-1, mir-7d, mir-20, mir-21, mir-26a, mir-192, mir-193, mir-206, mir-220, mir-290, mir-294, mir-329, mir-371, mir-373, or mir-409.

Other methods concern inhibiting cells in G1 phase by introducing into or providing to the cells an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is Let-7a, mir-1, mir-7d, mir-20, mir-21, mir-26a, mir-192, mir-193, mir-206, mir-220, mir-290, mir-294, mir-329, mir-371, mir-373, mir-409; or 2) at least one miRNA inhibitor corresponding to mir-108, mir-122, mir-124, mir-125a, mir-126, mir-128, mir-129, mir-137, mir-142, mir-146, mir-147, mir-195, mir-201, mir-297, mir-320, mir-325, mir-324-3p, mir-337, mir-371, mir-376b, or mir-409.

Also, there are methods of promoting cells to be in G2/M phase by introducing into or providing to the cells an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is mir-1, mir-7a, mir-7d, mir-7g, mir-20, mir-21, mir-26a, mir-145, mir-187, mir-192, mir-193, mir-206, mir-215, mir-220, mir-223, mir-294, mir-329, mir-371, mir-373, or mir-409; or 2) at least one miRNA inhibitor corresponding to mir-15a, mir-18, mir-122, mir-124, mir-126, mir-128, mir-129, mir-137, mir-146, mir-147, mir-195, mir-219, mir-337, or mir-371.

In other embodiments there are methods relating to inhibiting cells to be in G2/M phase by introducing into or providing to the cells an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of 1) at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is mir-15a, mir-18, mir-122, mir-124, mir-126, mir-128, mir-129, mir-137, mir-146, mir-147, mir-195, mir-219, mir-337, or mir-371; or 2) at least one miRNA inhibitor corresponding to mir-1, mir-7a, mir-7d, mir-7g, mir-20, mir-21, mir-26a, mir-145, mir-187, mir-192, mir-193, mir-206, mir-215, mir-220, mir-223, mir-294, mir-329, mir-371, mir-373, or mir-409.

The present invention also includes methods of increasing the number of cells with 2x or more DNA in the cell comprising introducing into or providing to the cells an effective amount of i) an miRNA inhibitor molecule or ii) a synthetic or nonsynthetic miRNA molecule that corresponds to an miRNA sequence. In certain embodiments the methods involves providing to or introducing into cells an effective amount of at least one nucleic acid molecule capable of being processed into a mature miRNA when it is inside the cell, wherein the mature miRNA is miR-1, miR-20, miR-21, miR-337, miR-345, or miR-373. The present invention is also concerned with reducing the number of cells with 2x (also referred to as 2N, where N is the number of sets of chromosomes) comprising providing to or introducing into cells an effective amount of an miRNA inhibitor corresponding to miR-1, miR-20, miR-21, miR-337, miR-345, or miR-373.

In certain embodiments, methods also include targeting an miRNA to modulate in a cell or organism. The term “targeting an miRNA to modulate” means a nucleic acid of the invention will be employed so as to modulate the selected miRNA. In some embodiments the modulation is achieved with a synthetic or non-synthetic miRNA that corresponds to the targeted miRNA, which effectively provides the targeted miRNA to the cell or organism (positive modulation). In other embodiments, the modulation is achieved with an miRNA inhibitor, which effectively inhibits the targeted miRNA in the cell or organism (negative modulation).

In some embodiments, the miRNA targeted to be modulated is an miRNA that affects a disease, condition, or pathway. In certain embodiments, the miRNA is targeted because a treatment can be provided by negative modulation of the targeted miRNA. In other embodiments, the miRNA is targeted because a treatment can be provided by positive modulation of the targeted miRNA.

In further embodiments of the invention, there is a step of obtaining a nucleic molecule of the invention that achieves negative modulation of the targeted miRNA.

Alternatively, in some cases there is a step of obtaining a nucleic molecule of the invention that achieves positive modulation of the targeted miRNA. Thus, it is contemplated that methods involve selecting and/or obtaining a synthetic miRNA, non-synthetic miRNA or an miRNA inhibitor (collectively "miRNA modulators") that corresponds to a targeted miRNA, such as one that is involved with, affects or is characteristic of a particular disease, condition, pathway, or factor in the pathway.

In certain methods of the invention, there is a further step of administering the selected miRNA modulator to a cell, tissue, organ, or organism (collectively "biological matter") in need of treatment related to modulation of the targeted miRNA or in need of the physiological or biological results discussed herein (such as with respect to a particular cellular pathway or result like decrease in cell viability). Consequently, in some methods of the invention there is a step of identifying a patient in need of treatment that can be provided by the miRNA modulator(s). It is contemplated that an effective amount of an miRNA modulator can be administered in some embodiments. In particular embodiments, there is a therapeutic benefit conferred on the biological matter, where a "therapeutic benefit" refers to an improvement in the one or more conditions or symptoms associated with a disease or condition or an improvement in the prognosis, duration, or status with respect to the disase. It is contemplated that a therapeutic benefit includes, but is not limited to, a decrease in pain, a decrease in morbidity, a decrease in a symptom. For example, with respect to cancer, it is contemplated that a therapeutic benefit can be inhibition of tumor growth, prevention of metastasis, reduction in number of metastases, inhibition of cancer cell proliferation, inhibition of cancer cell proliferation, induction of cell death in cancer cells, inhibition of angiogenesis near cancer cells, induction of apoptosis of cancer cells, reduction in pain, reduction in risk of recurrence, induction of chemo- or radiosensitivity in cancer cells, prolongation of life, and/or delay of death directly or indirectly related to cancer.

It is specifically contemplated that miRNA profiles for patients, particularly those suspected of having a particular disease or condition, can be generated by evaluating any of the miRNAs discussed in this application. The miRNA profile that is generated from the patient will be one that provides information regarding the particular disease or condition. In many embodiments, the miRNA profile is generated using the miRNA array discussed.

Furthermore, it is contemplated that the miRNA compositions may be provided as part of a therapy to a patient, in conjunction with traditional therapies or preventative agents. Moreover, it is contemplated that any method discussed in the context of therapy may be applied as preventatively, particularly in a patient identified to be potentially in need of the therapy or at risk of the condition or disease for which a therapy is needed.

In other embodiments, the invention concerns a method for inducing transformation in a cell comprising administering to the cell an effective amount of at least one miRNA selected from the group consisting of mir-192, mir-198, and mir-199. Alternatively, methods for preventing cell transformation may be achieved by administering to the cell an effective amount of at least one miRNA inhibitor of mir-192, mir-198, or mir-199.

In addition, methods of the invention concern employing one or more nucleic acids corresponding to an miRNA and a therapeutic drug. The nucleic acid can enhance the effect or efficacy of the drug, reduce any side effects or toxicity, modify its bioavailability, and/or decrease the dosage or frequency needed. In certain embodiments, the therapeutic drug is a cancer therapeutic. Consequently, in some embodiments, there is a method of treating cancer in a patient comprising administering to the patient the cancer therapeutic and an effective amount of at least one miRNA molecule that improves the efficacy of the cancer therapeutic or protects non-cancer cells. Furthermore, in some cases the miRNA molecule enhances the efficacy of the cancer therapeutic and is selected from the group consisting of ambi-miR-7100, mir-28, mir-101, mir-124, mir-125a, mir-126, mir-132, mir-136, mir-147, mir-155, mir-182, mir-186, mir-202, mir-206, mir-216, mir-221, mir-224, mir-291, mir-292-3p, mir-297, mir-302, mir-337, mir-372, mir-373, and mir-376b.

Cancer therapies also include a variety of combination therapies with both chemical and radiation based treatments. Combination chemotherapies include but are not limited to, for example, bevacizumab, cisplatin (CDDP), carboplatin, EGFR inhibitors (gefitinib and cetuximab), procarbazine, mechlorethamine, cyclophosphamide, camptothecin, COX-2 inhibitors (*e.g.*, celecoxib) ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin (adriamycin), bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, taxotere, gemcitabien, navelbine, farnesyl-protein transferase inhibitors, transplatinum, 5-fluorouracil, vincristin, vinblastin and methotrexate, or any analog or derivative variant of the foregoing.

Alternatively or additionally, the miRNA molecule in methods of the invention protects non-cancer cells from the cancer therapeutic and is selected from the group consisting of mir-16, mir-24, mir-30a-3p, mir-125b, mir-152, mir-194, mir-197, mir-214, and mir-331.

Generally, inhibitors of miRNAs can be given to achieve the opposite effect as compared to when nucleic acid molecules corresponding to the mature miRNA are given. Similarly, nucleic acid molecules corresponding to the mature miRNA can be given to achieve the opposite effect as compared to when inhibitors of the miRNA are given. For example, miRNA molecules that increase cell proliferation can be provided to cells to increase proliferation or inhibitors of such molecules can be provided to cells to decrease cell proliferation. The present invention contemplates these embodiments in the context of the different physiological effects observed with the different miRNA molecules and miRNA inhibitors disclosed herein. These include, but are not limited to, the following physiological effects: increase and decreasing cell proliferation, increasing or decreasing apoptosis, increasing transformation, increasing or decreasing cell viability, activating ERK, activating/inducing or inhibiting hTert, inhibit stimulation of Stat3, reduce or increase viable cell number, and increase or decrease number of cells at a particular phase of the cell cycle. Methods of the invention are generally contemplated to include providing or introducing one or more different nucleic acid molecules corresponding to one or more different miRNA molecules. It is contemplated that the following, at least the following, or at most the following number of different nucleic acid molecules may be provided or introduced: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or any range derivable therein. This also applies to the number of different miRNA molecules that can be provided or introduced into a cell.

The present invention also concerns kit containing compositions of the invention or compositions to implement methods of the invention. In some embodiments, kits can be used to evaluate one or more miRNA molecules. In certain embodiments, a kit contains, contains at least or contains at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more synthetic miRNA molecules or miRNA inhibitors, or any range and combination derivable therein. In some embodiments, there are kits for evaluating miRNA activity in a cell.

Kits may comprise components, which may be individually packaged or placed in a container, such as a tube, bottle, vial, syringe, or other suitable container means.

Individual components may also be provided in a kit in concentrated amounts; in some embodiments, a component is provided individually in the same concentration as it would be in a solution with other components. Concentrations of components may be provided as 1x, 2x, 5x, 10x, or 20x or more.

Kits for using synthetic miRNAs, nonsynthetic, and/or miRNA inhibitors of the invention for therapeutic, prognostic, or diagnostic applications are included as part of the invention. Specifically contemplated are any such molecules corresponding to any miRNA reported to influence biological activity, such as those discussed herein.

Negative and/or Positive Control synthetic miRNAs and/or miRNA inhibitors are included in some kit embodiments. The Control molecules can be used to verify transfection efficiency and/or control for transfection-induced changes in cells. .

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein and that different embodiments may be combined. It is specifically contemplated that any methods and compositions discussed herein with respect to miRNA molecules or miRNA may be implemented with respect to synthetic miRNAs to the extent the synthetic miRNA is exposed to the proper conditions to allow it to become a mature miRNA under physiological circumstances. The claims originally filed are contemplated to cover claims that are multiply dependent on any filed claim or combination of filed claims.

Any embodiment of the invention involving specific miRNAs by name is contemplated also to cover embodiments involving miRNAs whose sequences are at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% identical to the mature sequence of the specified miRNA.

Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

It is specifically contemplated that any embodiments described in the Examples section are included as an embodiment of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1 Overview of miRNA Expression and Activation. MiRNAs are transcribed as part of longer RNA molecules that can be as long as a thousand nucleotides (Lee, 2002). The RNAs are processed in the nucleus into hairpin RNAs of 70-100 nucleotides by the dsRNA-specific ribonuclease Drosha (Lee 2003) (FIG. 1). The hairpin RNAs are transported to the cytoplasm and digested by a second, double-strand specific ribonuclease called Dicer. The resulting 19-23mer miRNA is bound by a complex that is similar to or identical to the RNA-Induced Silencing Complex (RISC) that participates in RNA interference (Hutvagner, 2002). The complex-bound, single-stranded miRNA binds mRNAs with sequences that are significantly, though not completely, complementary to the miRNA. By a mechanism that is not fully understood, but that does not involve mRNA degradation, the bound mRNA is not translated, resulting in reduced expression of the corresponding gene.

FIG. 2. Methods for Introducing miRNAs into Cells. There are three basic methods for introducing miRNAs into cells. In the first, a DNA bearing a promoter upstream of a sequence encoding a miRNAs is introduced into cells where it is transcribed to produce an RNA molecule that includes the mature miRNA. Processing and uptake by the protein complex for miRNA-induced gene regulation results in the activation of the miRNA. This method suffers from inefficient introduction of the DNA construct into cells. In the second method, an siRNA-like dsRNA molecule, one of whose strands is identical to an active miRNA is introduced into cells where it is taken up by the protein complex for miRNA activation. This method provides efficient deliver, but often uptake of the unintended complementary RNA molecule. The third method, described herein, involves modifying the complementary strand so as to favor uptake and activation of the active strand of the synthetic miRNA construct.

FIG 3. Preferential Uptake of Active Strands in synthetic miRNAs of the invention. Reporter vectors with luciferase under the control of target sites for miR-33 or let-7 or the complementary strands of the afore-mentioned siRNAs. Co-transfection of synthetic miRNAs and reporter vectors followed by luciferase assay 24 hours post-transfection revealed miRNAs that are activated following transfection.

FIG 4. Synthetic miRNA Activity for various miRNAs. Synthetic miRNAs with siRNA and Pre-miR (5'amine) design were prepared and transfected into HeLa cells at 3 and 10 nM final concentration. The synthetic miRNAs were co-transfected with reporter vectors bearing target sites for the mature miRNAs. The expression of the luciferase reporter in co-transfected cells was measured twenty-four hours post-transfected and expressed in the figure as the reporter expression relative to cells co-transfeted with negative control synthetic miRNAs.

FIG 5. Synthetic miRNA Activity across Cell Types and Against Natural Targets. Synthetic miRNAs were tested for proper strand activation and cell-type specificity to ensure that the design is robust. Four different cell types were co-transfected with synthetic miRNA and associated active and complementary strand

strand activation. Panel A shows that different cell types respond similarly to synthetic miRNAs. Four different synthetic miRNAs were then transfected into various cell types and the expression levels of natural targets of the miRNAs were measured (Panel B).

FIG. 6. Schematic for screening with libraries of synthetic miRNAs or miRNA inhibitors. Synthetic miRNAs and/or miRNA inhibitors are distributed to wells of a microtiter plate. Transfection reagent and then cells are added to each well. At some time post-transfection, samples are evaluated for a phenotype. MiRNAs that induce a change that is significant relative to a negative control are selected for further study.

FIG. 7. Screen for miRNAs that affect cell proliferation. In 96-well plates, 8,000 HeLa cells were reverse transfected with miRNA inhibitors (5 pmoles) in triplicates using Ambion siPORT Neo-FX™. 72 hours post-transfection, cells were fixed with 4% paraformaldehyde, permiabilized with 0.1% Triton™X 100 and stained with propidium iodide to look at total cell number. The plates were scanned using the TTP labtech Acumen Explorer™. Morphology changes in cells inhibited for mir 31. HeLa cells were transfected with Anti-mir31 and cells were fixed and stained with anti-beta actin antibody and DAPI to visualize cell morphology changes in response to inhibition to mir-31 micro-RNA function.

FIG. 8. Screen for miRNAs that affect cell proliferation in A549 cells. Screen for miRNA involved in cell viability in A549 cells. In 96-well plates, 8,000 A549 cells were reverse transfected with miRNA inhibitors (5 pmoles) in triplicates using Ambion siPORT Neo-FX. 72 hours post-transfection cells were trypsinized and counted using the Guava™ cell counting instrument. Cell number was graphed and normalized to a gap inhibitor. In this figure, "mir1d" refers to mir-1-2.

FIG. 9. Screen for miRNAs that affect apoptosis in HeLa cells. Effects of miRNA inhibitors on caspase activity in HeLa. In 96-well plates, 8,000 HeLa cells were reverse transfected with miRNA inhibitors (5 pmoles) in triplicates using Ambion siPORT Neo-FX. 72 hours post-transfection cells were analyzed using caspase activity assay and normalized based on esterase activity assay. In this figure, "mir1d" refers to mir-1-2.

FIG. 10. miRNA Expression in Lung and Colon Cancer Patients. The miRNA expression profiles of tumor vs normal adjacent tissues were compared for lung and colon cancer patients. The miRNAs are provided in rows; the patients are presented in columns. Green in the heat map shows miRNAs that are down-regulated in the tumor sample relative to the normal adjacent tissue sample, and red shows miRNAs that are up-regulated in the tumor sample relative to the normal adjacent tissue sample.

FIG. 11. Validation of miRNA Array Expression Results in Lung Cancer Patients. Total RNA samples from two lung cancer patients were analyzed for expression of miR-16, miR-21, miR-143, miR-145, and let-7 using Northern analysis. The graphs show the relative abundance of each miRNA (ratio of tumor:NAT) from the array analysis and Northern phosphoimager analysis.

FIG. 12. Some miRNAs are differentially expressed in multiple cancer types. miRNA array analysis comparing tumor and normal adjacent tissues from patients with various types of cancer was used to identify miRNAs that are differentially expressed in cancer. The percentage of patients exhibiting up- or down-regulation of a given miRNA was calculated for each cancer type. The eight that were most often differentially expressed across sample types are presented.

FIG. 13. Shown are miRNAs having greater than 1.5-fold expression changes between both infected vs. uninfected and sensitive vs. insensitive. On the right is a cluster of the results from 2 arrays of each model.

FIG. 14. Differentially expressed miRNAs in 3 preconditioned mice relative to non-treated mice.

FIG. 15A-C. Synthetic miRNAs that decrease cell proliferation. A. BT549 and MCF12A (breast), HeLa (cervical) and 22 Rv1 (prostate) cells were evaluated for cell proliferation. **B.** TE354T and TE353SK (skin), BJ (skin), and A549 (lung) cells were examined for cell proliferation. **C.** CRL5826 and HTB-57 (lung), Jurkats (T cell), and primary T cells were evaluated for cell proliferation.

FIG. 16. Synthetic miRNAs that increase cell proliferation. HeLa (cervical), 22 Rv1 (prostate), TE354T and TE353SK (skin), BJ (skin), A549 (lung), Jurkats (T cell), primary T cells, CRL5826 and HTB-57 (lung) cells were evaluated for cell proliferation.

FIG. 17. miRNA inhibitors that reduce cell proliferation. 22 Rv1 (prostate), TE354T (skin), MCF12a (breast), and A549 (lung) cells were evaluated for cell proliferation.

FIG. 18. miRNA inhibitors that increase cell proliferation. 22 Rv1 (prostate), TE354T (skin), MCF12a (breast), and A549 (lung) cells were evaluated for cell proliferation.

FIG. 19. miRNAs that affect cell viability. Jurkats (T cell), primary T cells, HeLa (cervical) and A549 (lung) cells were evaluated for increases and decreases in cell viability.

FIG. 20. miRNAs that affect apoptosis. 22 Rv1 (prostate), TE354T (skin), Jurkats (T cell), and HeLa (cervical) cells were evaluated for increases and decreases in apoptosis.

FIG. 21. miRNAs that affect cell viability in the presence of a therapeutic. A549 (lung) cells were evaluated for increases and decreases in cell viability in the presence and absence of TRAIL or etoposide. HTB-57 and CRL5826 (lung) and HeLa (cervical) cells were evaluated for a reduction in cell viability in the absence and presence of etoposide.

FIG. 22. miRNAs that affect cell cycle. BJ (skin) and HeLa (cervical) cells were evaluated for increases or decreases in the number of cells at certain phases of the cell cycle (G1, S, G2/M, DNA replication).

FIG. 23. Phenotypes of miRNAs with similar sequences. Comparison of related sequences and their effects on cell proliferation.

FIG. 24. Genes associated with hTert regulation and miRNA sequences predicted to modulate their expression.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention is directed to compositions and methods relating to preparation and characterization of miRNAs, as well as use of miRNAs for therapeutic, prognostic, and diagnostic applications. To overcome the problem with previous inefficient plasmid-based systems for introducing miRNA into cells, the inventors developed small, partially double-stranded RNAs that can be delivered with high efficiency to both immortalized and primary cells. The small RNAs have the same functional activities as endogenously expressed miRNAs. Because the small RNAs can be delivered to cells with much higher efficiency than can plasmids, they induce a much stronger phenotype that is easier to detect and quantify, making it possible to identify many of the functions of miRNAs in cells.

The inventors have also created a library of the small, double-stranded RNA molecules that can be used to introduce miRNAs into cells, as well as a library of antisense molecules that inhibit the activities of known miRNAs that are present in cells. These libraries have been used to sequentially up- or down-regulate one or more miRNAs in cells to identify those miRNAs that are critical for cellular processes like cell cycle, apoptosis, differentiation, viability, angiogenesis, metabolism, and other processes with therapeutic potential. miRNAs that regulate the expression of important genes like p53, MYC, and RAS are also being identified and characterized to further pinpoint miRNAs that might provide important intervention points for treating disease. For example, let-7 has been shown to be involved with RAS. See Johnson *et al.*, 2005. These processes of serially modulating miRNA activities and assaying for cellular phenotypes are collectively referred to as miRNA functional screening.

I. miRNA Molecules

MicroRNA molecules ("miRNAs") are generally 21 to 22 nucleotides in length, though lengths of 17 and up to 25 nucleotides have been reported. The miRNAs are each processed from a longer precursor RNA molecule ("precursor miRNA"). Precursor miRNAs are transcribed from non-protein-encoding genes. The precursor miRNAs have two regions of complementarity that enables them to form a stem-loop- or fold-back-like structure, which is cleaved by an enzyme called Dicer in animals. Dicer is ribonuclease III-like nuclease. The processed miRNA is typically a portion of the stem.

The processed miRNA (also referred to as "mature miRNA") become part of a large complex to down-regulate a particular target gene. Examples of animal miRNAs include those that imperfectly basepair with the target, which halts translation (Olsen *et al.*, 1999; Seggerson *et al.*, 2002). SiRNA molecules also are processed by Dicer, but from a long, double-stranded RNA molecule. SiRNAs are not naturally found in animal cells, but they can function in such cells in a RNA-induced silencing complex

(RISC) to direct the sequence-specific cleavage of an mRNA target (Denli *et al.*, 2003).

Synthetic miRNAs

miRNAs are apparently active in the cell when the mature, single-stranded RNA is bound by a protein complex that regulates the translation of mRNAs that hybridize to the miRNA. Introducing exogenous RNA molecules that affect cells in the same way as endogenously expressed miRNAs requires that a single-stranded RNA molecule of the same sequence as the endogenous mature miRNA be taken up by the protein complex that facilitates translational control. A variety of RNA molecule designs have been evaluated. Three general designs that maximize uptake of the desired single-stranded miRNA by the miRNA pathway have been identified. An RNA molecule with an miRNA sequence having at least one of the three designs is referred to as a synthetic miRNA.

Synthetic miRNAs of the invention comprise, in some embodiments, two RNA molecules wherein one RNA is identical to a naturally occurring, mature miRNA. The RNA molecule that is identical to a mature miRNA is referred to as the active strand. The second RNA molecule, referred to as the complementary strand, is at least partially complementary to the active strand. The active and complementary strands are hybridized to create a double-stranded RNA, called the synthetic miRNA, that is similar to the naturally occurring miRNA precursor that is bound by the protein complex immediately prior to miRNA activation in the cell. Maximizing activity of the synthetic miRNA requires maximizing uptake of the active strand and minimizing uptake of the complementary strand by the miRNA protein complex that regulates gene expression at the level of translation. The molecular designs that provide optimal miRNA activity involve modifications to the complementary strand.

Two designs incorporate chemical modifications in the complementary strand. The first modification involves creating a complementary RNA with a chemical group other than a phosphate or hydroxyl at its 5′ terminus. The presence of the 5′ modification apparently eliminates uptake of the complementary strand and subsequently favors uptake of the active strand by the miRNA protein complex. The

5′ modification can be any of a variety of molecules including NH_2, $NHCOCH_3$, biotin, and others.

The second chemical modification strategy that significantly reduces uptake of the complementary strand by the miRNA pathway is incorporating nucleotides with sugar modifications in the first 2-6 nucleotides of the complementary strand. It should be noted that the sugar modifications consistent with the second design strategy can be coupled with 5′ terminal modifications consistent with the first design strategy to further enhance synthetic miRNA activities.

The third synthetic miRNA design involves incorporating nucleotides in the 3′ end of the complementary strand that are not complementary to the active strand. Hybrids of the resulting active and complementary RNAs are very stable at the 3′ end of the active strand but relatively unstable at the 5′ end of the active strand. Studies with siRNAs indicate that 5′ hybrid stability is a key indicator of RNA uptake by the protein complex that supports RNA interference, which is at least related to the miRNA pathwy in cells. The inventors have found that the judicious use of mismatches in the complementary RNA strand significantly enhances the activity of the synthetic miRNA.

<u>MiRNA Libraries</u>

A key application for the synthetic miRNAs is the identification of cellular functions for individual or groups of miRNAs. The inventors have created a library of synthetic miRNAs that can be used to sequentially introduce each of the known miRNAs into cultured cells (FIG. 6). Cell populations with each of the different synthetic miRNAs can then be assayed to identify miRNAs whose presence induces a cellular phenotype.

The inventors have created a library of antisense molecules that inhibit miRNA activity. The miRNA inhibitors are used to serially inhibit the activities of miRNAs in cells to identify miRNAs whose absence induces a cellular phenotype.

The number of different synthetic miRNAs or miRNA inhibitors in the libraries is variable. It is contemplated that there may be, be at least, or be at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 31, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 6000, 7000, 8000, 9000, 10000 or more, or any range derivable therein, different miRNA-specific molecules in the library. In specific embodiments, libraries have between 5 and 1000 different miRNA-specific molecules, between 20 and 500 different miRNA-specific molecules, between 50 and 250 different miRNA-specific molecules, or between 100 and 225 different miRNA-specific molecules. "Different" miRNA-specific molecules refers to nucleic acids that are specific to miRNAs with different sequences.

Synthetic miRNAs are contemplated to be made primarily of RNA, though in some embodiments, they may be RNA, nucleotide analogs, DNA, or any combination of DNA, RNA, nucleotide analogs, and PNAs.

As suggested above, it is contemplated that libraries of the invention may be specific for one or more miRNAs. In embodiments of the invention, a library has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 31, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 6000, 7000, 8000, 9000, 10000 or more, or any range derivable therein, different miRNAs or miRNA inhibitors. Accordingly, it is understood that the library contains one or more nucleic acids for these different miRNAs. In specific embodiments, the library is specific to human miRNAs, though libraries for multiple organisms are contemplated.

RNA molecules of the invention have miRNA regions or complementary regions. In specific embodiments, a synthetic miRNA or miRNA inhibitor has a sequence or complementary sequence that derives from any of SEQ ID NOs: 1-805, inclusive. It is particularly contemplated that synthetic nucleic acid molecules of the invention may be derived from any of the mature miRNA sequences in SEQ ID NOs:1-805 or their complement.

As discussed above, miRNAs are processed from a precursor molecule. In certain embodiments, the specific length of a mature miRNA is unknown. It is contemplated that versions of the synthetic miRNA and miRNA inhibitor libraries will include sequence that extends at least 1 to 5 nucleotides of coding sequence upstream and/or downstream of the predicted miRNA sequence. In some embodiments, molecules have up to 1, 2, 3, 4, 5, 6, 7, or more contiguous nucleotides, or any range derivable therein, that flank the sequence encoding the predominant processed miRNA on one or both sides (5′ and/or 3′ end).

The present invention concerns methods for creating functional profile for all of the known miRNAs. The term "functional profile" refers to a set of data regarding the cellular phenotypes that result from introducing and inhibiting miRNAs in cells using synthetic miRNA and miRNA inihibitor libraries. Functional profiles for individual miRNAs will enable identification of miRNAs with therapeutic or diagnostic potential. For instance, a functional profile for a miRNA might reveal that its absence leads to uncontrolled cell proliferation and an inability to induce apoptosis following DNA damage. Furthermore, the expression of p53 correlates with whether the miRNA is being up-regulated with a synthetic miRNA or down-regulated with a miRNA inhibitor. Based on its ties to cell proliferation, apoptosis, and p53 expression, this miRNA might be a target for cancer therapeutics.

In certain embodiments, methods concern identifying miRNAs indicative of a disease or condition by detecting a correlation between the activity of particular miRNAs and cellular phenotypes that coincide with a disease or condition.

Libraries of the invention can contain miRNA sequences from any organism having miRNAs, specifically including but not limited to, mammals such as humans, mice, and rats. Specifically contemplated are libraries having, having at least, or having at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7400, 7500, 7600, 7700, 7800, 7900, 8000, 8100, 8200, 8300, 8400, 8500, 8600, 8700, 8800, 8900, 9000, 9100, 9200, 9300, 9400, 9500, 9600, 9700, 9800, 9900, 10000 or more different synthetic miRNAs and/or miRNA inhibitors (that is, miRNA-specific molecules having different sequences derived from different miRNA genes). Specifically contemplated are such libraries described in the previous sentence with respect to any of SEQ ID NOs:1-805, particularly those corresponding to miRNA sequence s(mature sequence) or the complement thereof.

A. Nucleic Acids

The present invention concerns nucleic acid molecules that can introduce or inhibit miRNAs in cultured cells. The nucleic acids may have been produced in cells or in vitro by purified enzymes though they are preferentially produced by chemical synthesis. They may be crude or purified. The term "miRNA," unless otherwise indicated, refers to the processed RNA, after it has been cleaved from its precursor. Table 1 indicates which SEQ ID NO corresponds to the particular precursor sequence of an miRNA and what sequences within the SEQ ID NO correspond to the mature sequence. The name of the miRNA is often abbreviated and referred to without the prefix and will be understood as such, depending on the context. Unless otherwise indicated, miRNAs referred to in the application are human sequences identified as mir-X or let-X, where X is a number and/or letter.

Table 1
Human miRNA Sequences

miRNA name	Precursor	Processed Sequence Relative to Precursor
hsa-mir-1-2	SEQ ID NO:1	53-73
hsa-mir-1-1	SEQ ID NO:2	46-66
hsa-let-7a-1	SEQ ID NO:3	6-27
hsa-let-7a-2	SEQ ID NO:4	5-26
hsa-let-7a-3	SEQ ID NO:5	4-25
hsa-let-7b	SEQ ID NO:6	6-27
hsa—let-7c	SEQ ID NO:7	11-32
hsa-let-7d	SEQ ID NO:8	8-28
hsa-let-7e	SEQ ID NO:9	8-28
hsa-let-7f-1	SEQ ID NO:10	7-28
hsa-let-7f-2	SEQ ID NO:11	8-29
hsa-mir-7-1	SEQ ID NO:12	24-44
hsa-mir-7-2	SEQ ID NO:13	32-52
hsa-mir-7-3	SEQ ID NO:14	31-51
hsa-let-7g	SEQ ID NO:15	5-25
hsa-let-7i	SEQ ID NO:16	6-24
hsa-mir-9-1	SEQ ID NO:17	16-38 and/or 56-76
hsa-mir-9-2	SEQ ID NO:18	16-38 and/or 54-74
hsa-mir-9-3	SEQ ID NO:19	16-38 and/or 56-76
hsa-mir-10a	SEQ ID NO:20	22-44
hsa-mir-10b	SEQ ID NO:21	27-48
hsa-mir-15a	SEQ ID NO:22	14-35
hsa-mir-15b	SEQ ID NO:23	20-41
hsa-mir-16-1	SEQ ID NO:24	14-35
hsa-mir-16-2	SEQ ID NO:25	10-31
hsa-mir-17	SEQ ID NO:26	14-37 and/or 51-70
hsa-mir-18	SEQ ID NO:27	6-27
hsa-mir-19a	SEQ ID NO:28	49-71
hsa-mir-19b-1	SEQ ID NO:29	54-76
hsa-mir-19b-2	SEQ ID NO:30	62-84
hsa-mir-20	SEQ ID NO:31	8-29
hsa-mir-21	SEQ ID NO:32	8-29
hsa-mir-22	SEQ ID NO:33	53-74
hsa-mir-23a	SEQ ID NO:34	45-65
hsa-mir-23b	SEQ ID NO:35	58-80
hsa-mir-24-1	SEQ ID NO:36	6-28 and/or 44-65
hsa-mir-24-2	SEQ ID NO:37	50-71
hsa-mir-25	SEQ ID NO:38	52-73
hsa-mir-26a-1	SEQ ID NO:39	10-31
hsa-mir-26b	SEQ ID NO:40	12-32
hsa-mir-26a-2	SEQ ID NO:41	14-35
hsa-mir-27a	SEQ ID NO:42	51-72

miRNA name	Precursor	Processed Sequence Relative to Precursor
hsa-mir-27b	SEQ ID NO:43	61-80
hsa-mir-28	SEQ ID NO:44	14-35
hsa-mir-29a	SEQ ID NO:45	41-62
hsa-mir-29b-1	SEQ ID NO:46	51-70
hsa-mir-29b-2	SEQ ID NO:47	52-71
hsa-mir-29c	SEQ ID NO:48	54-75
hsa-mir-30a	SEQ ID NO:49	47-68
hsa-mir-30c-2	SEQ ID NO:50	7-29
hsa-mir-30d	SEQ ID NO:51	6-27
hsa-mir-30b	SEQ ID NO:52	17-37
hsa-mir-30c-1	SEQ ID NO:53	17-39
hsa-mir-30e	SEQ ID NO:54	2-21
hsa-mir-31	SEQ ID NO:55	9-29
hsa-mir-32	SEQ ID NO:56	6-26
hsa-mir-33	SEQ ID NO:57	6-24
hsa-mir-34a	SEQ ID NO:58	22-43
hsa-mir-34b	SEQ ID NO:59	14-35
hsa-mir-34c	SEQ ID NO:60	13-34
hsa-mir-92-1	SEQ ID NO:61	48-69
hsa-mir-92-2	SEQ ID NO:62	48-69
hsa-mir-93	SEQ ID NO:63	12-33
hsa-mir-95	SEQ ID NO:64	49-70
hsa-mir-96	SEQ ID NO:65	9-30
hsa-mir-98	SEQ ID NO:66	2-23
hsa-mir-99a	SEQ ID NO:67	13-34
hsa-mir-99b	SEQ ID NO:68	7-28
hsa-mir-100	SEQ ID NO:69	13-34
hsa-mir-101-1	SEQ ID NO:70	47-68
hsa-mir-101-2	SEQ ID NO:71	49-70
hsa-mir-103-2	SEQ ID NO:72	48-70
hsa-mir-103-1	SEQ ID NO:73	48-70
hsa-mir-105-1	SEQ ID NO:74	13-32
hsa-mir-105-2	SEQ ID NO:75	13-32
hsa-mir-106a	SEQ ID NO:76	13-36
hsa-mir-106b	SEQ ID NO:77	12-32
hsa-mir-107	SEQ ID NO:78	50-72
hsa-mir-122a	SEQ ID NO:79	15-37
hsa-mir-124a-1	SEQ ID NO:80	52-73
hsa-mir-124a-2	SEQ ID NO:81	61-82
hsa-mir-124a-3	SEQ ID NO:82	52-73
hsa-mir-125b-1	SEQ ID NO:83	15-36
hsa-mir-125a	SEQ ID NO:84	15-37
hsa-mir-125b-2	SEQ ID NO:85	17-38
hsa-mir-126	SEQ ID NO:86	15-35 and/or 52-72
hsa-mir-127	SEQ ID NO:87	57-78

miRNA name	Precursor	Processed Sequence Relative to Precursor
hsa-mir-128a	SEQ ID NO:88	50-71
hsa-mir-128b	SEQ ID NO:89	52-73
hsa-mir-129-2	SEQ ID NO:90	15-35
hsa-mir-130a	SEQ ID NO:91	55-74
hsa-mir-130b	SEQ ID NO:92	51-72
hsa-mir-132	SEQ ID NO:93	59-80
hsa-mir-133a-1	SEQ ID NO:94	54-75
hsa-mir-133a-2	SEQ ID NO:95	60-81
hsa-mir-133b	SEQ ID NO:96	67-87
hsa-mir-134	SEQ ID NO:97	8-28
hsa-mir-135a-1	SEQ ID NO:98	17-39
hsa-mir-135a-2	SEQ ID NO:99	23-45
hsa-mir-135b	SEQ ID NO:100	16-37
hsa-mir-136	SEQ ID NO:101	15-37
hsa-mir-137	SEQ ID NO:102	60-81
hsa-mir-138-2	SEQ ID NO:103	10-26
hsa-mir-138-1	SEQ ID NO:104	23-39
hsa-mir-139	SEQ ID NO:105	7-24
hsa-mir-140	SEQ ID NO:106	24-44
hsa-mir-141	SEQ ID NO:107	60-80
hsa-mir-142	SEQ ID NO:108	16-35 and/or 52-74
hsa-mir-143	SEQ ID NO:109	61-82
hsa-mir-144	SEQ ID NO:110	52-73
hsa-mir-145	SEQ ID NO:111	16-39
hsa-mir-146	SEQ ID NO:112	21-42
hsa-mir-147	SEQ ID NO:113	47-66
hsa-mir-148a	SEQ ID NO:114	44-65
hsa-mir-148b	SEQ ID NO:115	63-84
hsa-mir-149	SEQ ID NO:116	15-36
hsa-mir-150	SEQ ID NO:117	16-37
hsa-mir-151	SEQ ID NO:118	46-67
hsa-mir-152	SEQ ID NO:119	54-74
hsa-mir-153-1	SEQ ID NO:120	54-73
hsa-mir-153-2	SEQ ID NO:121	53-72
hsa-mir-154	SEQ ID NO:122	15-36
hsa-mir-155	SEQ ID NO:123	4-25
hsa-mir-181a	SEQ ID NO:124	39-61
hsa-mir-181b-1	SEQ ID NO:125	36-59
hsa-mir-181c	SEQ ID NO:126	27-48
hsa-mir-181b-2	SEQ ID NO:127	16-39
hsa-mir-182	SEQ ID NO:128	23-44 and/or 67-87
hsa-mir-183	SEQ ID NO:129	27-49
hsa-mir-184	SEQ ID NO:130	53-74
hsa-mir-185	SEQ ID NO:131	15-32
hsa-mir-186	SEQ ID NO:132	15-37

miRNA name	Precursor	Processed Sequence Relative to Precursor
hsa-mir-187	SEQ ID NO:133	71-91
hsa-mir-188	SEQ ID NO:134	15-36
hsa-mir-190	SEQ ID NO:135	15-36
hsa-mir-191	SEQ ID NO:136	16-37
hsa-mir-192	SEQ ID NO:137	24-44
hsa-mir-193	SEQ ID NO:138	55-75
hsa-mir-194-1	SEQ ID NO:139	15-36
hsa-mir-194-2	SEQ ID NO:140	15-36
hsa-mir-195	SEQ ID NO:141	15-35
hsa-mir-196-1	SEQ ID NO:142	7-27
hsa-mir-196-2	SEQ ID NO:143	25-45
hsa-mir-197	SEQ ID NO:144	48-69
hsa-mir-198	SEQ ID NO:145	6-24
hsa-mir-199a-1	SEQ ID NO:146	6-28 and/or 46-67
hsa-mir-199a-2	SEQ ID NO:147	31-53 and/or 69-90
hsa-mir-199b	SEQ ID NO:148	26-48
hsa-mir-200b	SEQ ID NO:149	54-77
hsa-mir-200c	SEQ ID NO:150	45-66
hsa-mir-200a	SEQ ID NO:151	54-75
hsa-mir-203	SEQ ID NO:152	65-86
hsa-mir-204	SEQ ID NO:153	33-54
hsa-mir-205	SEQ ID NO:154	34-55
hsa-mir-206	SEQ ID NO:155	53-74
hsa-mir-208	SEQ ID NO:156	44-65
hsa-mir-210	SEQ ID NO:157	66-86
hsa-mir-211	SEQ ID NO:158	26-47
hsa-mir-212	SEQ ID NO:159	71-91
hsa-mir-213	SEQ ID NO:160	24-46 and/or 64-85
hsa-mir-214	SEQ ID NO:161	71-91
hsa-mir-215	SEQ ID NO:162	27-47
hsa-mir-216	SEQ ID NO:163	19-39
hsa-mir-217	SEQ ID NO:164	35-58
hsa-mir-218-1	SEQ ID NO:165	25-45
hsa-mir-218-2	SEQ ID NO:166	25-45
hsa-mir-219-1	SEQ ID NO:167	21-41
hsa-mir-219-2	SEQ ID NO:168	19-39
hsa-mir-220	SEQ ID NO:169	23-43
hsa-mir-221	SEQ ID NO:170	65-87
hsa-mir-222	SEQ ID NO:171	69-92
hsa-mir-223	SEQ ID NO:172	68-88
hsa-mir-224	SEQ ID NO:173	8-30
hsa-mir-296	SEQ ID NO:174	14-34
hsa-mir-299	SEQ ID NO:175	7-28
hsa-mir-301	SEQ ID NO:176	51-73
hsa-mir-302	SEQ ID NO:177	44-66

miRNA name	Precursor	Processed Sequence Relative to Precursor
hsa-mir-320	SEQ ID NO:178	48-70
hsa-mir-321	SEQ ID NO:179	10-30
hsa-mir-323	SEQ ID NO:180	50-71
hsa-mir-324	SEQ ID NO:181	16-38 and/or 51-72
hsa-mir-326	SEQ ID NO:182	60-79
hsa-mir-328	SEQ ID NO:183	48-69
hsa-mir-330	SEQ ID NO:184	57-79
hsa-mir-331	SEQ ID NO:185	61-81
hsa-mir-335	SEQ ID NO:186	16-38
hsa-mir-337	SEQ ID NO:187	56-78
hsa-mir-338	SEQ ID NO:188	42-64
hsa-mir-339	SEQ ID NO:189	15-35
hsa-mir-340	SEQ ID NO:190	58-80
hsa-mir-342	SEQ ID NO:191	61-84
hsa-mir-345	SEQ ID NO:573	17-37
hsa-mir-346	SEQ ID NO:574	4-26
hsa-mir-367	SEQ ID NO:575	44-65
hsa-mir-368	SEQ ID NO:576	44-65
hsa-mir-369	SEQ ID NO:577	44-64
hsa-mir-370	SEQ ID NO:578	48-68
hsa-mir-371	SEQ ID NO:579	44-64
hsa-mir-372	SEQ ID NO:580	42-64
hsa-mir-373	SEQ ID NO:581	44-66
hsa-mir-374	SEQ ID NO:582	12-33
hsa-mir-375	SEQ ID NO:677	40-61
hsa-mir-376a	SEQ ID NO:678	44-64
hsa-mir-377	SEQ ID NO:679	45-66
hsa-mir-378	SEQ ID NO:680	5-26 and 44-65
hsa-mir-379	SEQ ID NO:681	6-24
hsa-mir-380	SEQ ID NO:682	5-26 and 40-61
hsa-mir-381	SEQ ID NO:683	49-70
hsa-mir-382	SEQ ID NO:684	11-32
hsa-mir-383	SEQ ID NO:685	7-28
hsa-mir-384	SEQ ID NO:686	57-76
hsa-mir-422a	SEQ ID NO:687	11-32
hsa-mir-423	SEQ ID NO:688	53-74
hsa-mir-424	SEQ ID NO:689	11-32
hsa-mir-425	SEQ ID NO:690	55-75
hsa-mir-448	SEQ ID NO:691	71-92
hsa-mir-429	SEQ ID NO:692	51-72
hsa-mir-449	SEQ ID NO:693	16-37
hsa-mir-450-1	SEQ ID NO:694	17-38
hsa-mir-450-2	SEQ ID NO:704	22-43
hsa-mir-451	SEQ ID NO:705	17-39
hsa-mir-452	SEQ ID NO:706	17-38

miRNA name	Precursor	Processed Sequence Relative to Precursor
hsa-mir-453	SEQ ID NO:707	43-64
hsa-mir-455	SEQ ID NO:708	16-37
hsa-mir-483	SEQ ID NO:709	48-70
hsa-mir-484	SEQ ID NO:710	2-23
hsa-mir-485	SEQ ID NO:711	9-30
hsa-mir-486	SEQ ID NO:712	4-25
hsa-mir-487	SEQ ID NO:713	49-70
hsa-mir-488	SEQ ID NO:714	14-34
hsa-mir-489	SEQ ID NO:715	51-73
hsa-mir-490	SEQ ID NO:716	76-97
hsa-mir-491	SEQ ID NO:717	16-38
hsa-mir-492	SEQ ID NO:718	30-52
hsa-mir-493	SEQ ID NO:719	16-37
hsa-mir-494	SEQ ID NO:720	48-71
hsa-mir-495	SEQ ID NO:721	50-72
hsa-mir-496	SEQ ID NO:722	61-77
hsa-mir-497	SEQ ID NO:723	24-44
hsa-mir-498	SEQ ID NO:724	34-56
hsa-mir-499	SEQ ID NO:725	33-55
hsa-mir-500	SEQ ID NO:726	52-73
hsa-mir-501	SEQ ID NO:727	14-35
hsa-mir-502	SEQ ID NO:728	1-21
hsa-mir-503	SEQ ID NO:729	6-28
hsa-mir-504	SEQ ID NO:730	13-33
hsa-mir-505	SEQ ID NO:731	52-73
hsa-mir-506	SEQ ID NO:732	71-91
hsa-mir-507	SEQ ID NO:733	56-76
hsa-mir-508	SEQ ID NO:734	61-83
hsa-mir-509	SEQ ID NO:735	55-77
hsa-mir-510	SEQ ID NO:736	10-32
hsa-mir-511-1	SEQ ID NO:737	16-36
hsa-mir-511-2	SEQ ID NO:738	16-36
hsa-mir-512-1	SEQ ID NO:739	14-36
hsa-mir-512-2	SEQ ID NO:740	20-42
hsa-mir-513-1	SEQ ID NO:741	37-58
hsa-mir-513-2	SEQ ID NO:742	36-57
hsa-mir-514-1	SEQ ID NO:743	39-58
hsa-mir-514-2	SEQ ID NO:744	39-58
hsa-mir-514-3	SEQ ID NO:745	39-58
hsa-mir-515-1	SEQ ID NO:746	14-37
hsa-mir-515-2	SEQ ID NO:747	14-37
hsa-mir-516-1	SEQ ID NO:748	61-78
hsa-mir-516-2	SEQ ID NO:749	61-78
hsa-mir-516-3	SEQ ID NO:750	15-37
hsa-mir-516-4	SEQ ID NO:751	15-37

miRNA name	Precursor	Processed Sequence Relative to Precursor
hsa-mir-517a	SEQ ID NO:752	15-36
hsa-mir-517b	SEQ ID NO:753	6-27
hsa-mir-517c	SEQ ID NO:754	20-41
hsa-mir-518a-1	SEQ ID NO:755	14-34
hsa-mir-518a-2	SEQ ID NO:756	15-34
hsa-mir-518b	SEQ ID NO:757	51-72
hsa-mir-518c	SEQ ID NO:758	24-46
hsa-mir-518d	SEQ ID NO:759	16-36
hsa-mir-518e	SEQ ID NO:760	54-75
hsa-mir-518f	SEQ ID NO:761	16-38
hsa-mir-519a-1	SEQ ID NO:762	15-38
hsa-mir-519a-2	SEQ ID NO:763	54-78
hsa-mir-519b	SEQ ID NO:764	13-36
hsa-mir-519c	SEQ ID NO:765	16-39
hsa-mir-519d	SEQ ID NO:766	54-76
hsa-mir-519e	SEQ ID NO:767	14-35
hsa-mir-520a	SEQ ID NO:768	15-35
hsa-mir-520b	SEQ ID NO:769	41-61
hsa-mir-520c	SEQ ID NO:770	16-36
hsa-mir-520d	SEQ ID NO:771	15-37
hsa-mir-520e	SEQ ID NO:772	54-74
hsa-mir-520f	SEQ ID NO:773	55-76
hsa-mir-520g	SEQ ID NO:774	55-78
hsa-mir-520h	SEQ ID NO:775	55-76
hsa-mir-521-1	SEQ ID NO:776	54-75
hsa-mir-521-2	SEQ ID NO:777	54-75
hsa-mir-522	SEQ ID NO:778	16-39
hsa-mir-523	SEQ ID NO:779	16-39
hsa-mir-524	SEQ ID NO:780	16-37
hsa-mir-525	SEQ ID NO:781	15-35
hsa-mir-526a-1	SEQ ID NO:782	15-35
hsa-mir-526a-2	SEQ ID NO:783	7-27
hsa-mir-526b	SEQ ID NO:784	14-37
hsa-mir-527	SEQ ID NO:785	14-34
ambi-mir-7100	SEQ ID NO:803	
mir-526b*	SEQ ID NO:804	
mir-520a*	SEQ ID NO:805	

Table 2
Mouse miRNA Sequences

miRNA name	Precursor	Processed Sequence Relative to Precursor
mmu-mir-1-1	SEQ ID NO:192	49-69
mmu-mir-1-2	SEQ ID NO:193	47-67

miRNA name	**Precursor**	**Processed Sequence Relative to Precursor**
mmu-let-7g	SEQ ID NO:194	7-27
mmu-let-7i	SEQ ID NO:195	6-24
mmu-let-7d	SEQ ID NO:196	16-36 + 70-91
mmu-let-7a-1	SEQ ID NO:197	13-34
mmu-let-7a-2	SEQ ID NO:198	17-38
mmu-let-7b	SEQ ID NO:199	7-28
mmu-let-7c-1	SEQ ID NO:200	16-37
mmu-let-7c-2	SEQ ID NO:201	14-35
mmu-let-7e	SEQ ID NO:202	15-35
mmu-let-7f-1	SEQ ID NO:203	8-29
mmu-let-7f-2	SEQ ID NO:204	8-29
mmu-mir-7-1	SEQ ID NO:205	24-44
mmu-mir-7-2	SEQ ID NO:206	19-39
mmu-mir-7b	SEQ ID NO:207	30-50
mmu-mir-9-2	SEQ ID NO:208	8-30 and/or 46-66
mmu-mir-9-1	SEQ ID NO:209	16-38 and/or 56-76
mmu-mir-9-3	SEQ ID NO:210	16-38 and/or 56-76
mmu-mir-10b	SEQ ID NO:211	7-28
mmu-mir-10a-1	SEQ ID NO:212	22-44
mmu-mir-10a-2	SEQ ID NO:213	22-44
mmu-mir-15b	SEQ ID NO:214	4-25
mmu-mir-15a	SEQ ID NO:215	15-36
mmu-mir-16-1	SEQ ID NO:216	16-37
mmu-mir-16-2	SEQ ID NO:217	17-38
mmu-mir-17	SEQ ID NO:218	14-37 and/or 51-70
mmu-mir-18	SEQ ID NO:219	17-38
mmu-mir-19b-2	SEQ ID NO:220	54-76
mmu-mir-19a	SEQ ID NO:221	49-71
mmu-mir-19b-1	SEQ ID NO:222	54-76
mmu-mir-20	SEQ ID NO:223	27-49
mmu-mir-21	SEQ ID NO:224	18-39
mmu-mir-22	SEQ ID NO:225	57-78
mmu-mir-23b	SEQ ID NO:226	46-68
mmu-mir-23a	SEQ ID NO:227	46-66
mmu-mir-24-1	SEQ ID NO:228	6-28 and/or 44-65
mmu-mir-24-2	SEQ ID NO:229	61-82
mmu-mir-25	SEQ ID NO:230	52-73
mmu-mir-26a-1	SEQ ID NO:231	16-37
mmu-mir-26b	SEQ ID NO:232	15-36
mmu-mir-26a-2	SEQ ID NO:233	14-35
mmu-mir-27b	SEQ ID NO:234	49-68
mmu-mir-27a	SEQ ID NO:235	56-76
mmu-mir-28	SEQ ID NO:236	14-35
mmu-mir-29b-1	SEQ ID NO:237	47-68
mmu-mir-29a	SEQ ID NO:238	53-74
mmu-mir-29c	SEQ ID NO:239	54-75

miRNA name	Precursor	Processed Sequence Relative to Precursor
mmu-mir-29b-2	SEQ ID NO:240	52-73
mmu-mir-30a	SEQ ID NO:241	47-68
mmu-mir-30b	SEQ ID NO:242	2-22
mmu-mir-30e	SEQ ID NO:243	2-21
mmu-mir-30c-1	SEQ ID NO:244	17-39
mmu-mir-30c-2	SEQ ID NO:245	14-36
mmu-mir-30d	SEQ ID NO:246	12-33
mmu-mir-31	SEQ ID NO:247	28-49
mmu-mir-32	SEQ ID NO:248	6-26
mmu-mir-33	SEQ ID NO:249	6-24
mmu-mir-34c	SEQ ID NO:250	13-35
mmu-mir-34b	SEQ ID NO:251	13-35
mmu-mir-34a	SEQ ID NO:252	20-42
mmu-mir-92-2	SEQ ID NO:253	55-75
mmu-mir-92-1	SEQ ID NO:254	50-70
mmu-mir-93	SEQ ID NO:255	15-37
mmu-mir-96	SEQ ID NO:256	24-46
mmu-mir-98	SEQ ID NO:257	2-23
mmu-mir-99a	SEQ ID NO:258	6-25
mmu-mir-99b	SEQ ID NO:259	7-28
mmu-mir-100	SEQ ID NO:260	13-34
mmu-mir-101	SEQ ID NO:261	38-57
mmu-mir-101b	SEQ ID NO:262	61-82
mmu-mir-103-1	SEQ ID NO:263	52-74
mmu-mir-103-2	SEQ ID NO:264	52-74
mmu-mir-106a	SEQ ID NO:265	5-26
mmu-mir-106b	SEQ ID NO:266	12-32
mmu-mir-107	SEQ ID NO:267	52-74
mmu-mir-122a	SEQ ID NO:268	6-28
mmu-mir-124a-3	SEQ ID NO:269	43-64
mmu-mir-124a-1	SEQ ID NO:270	52-73
mmu-mir-124a-2	SEQ ID NO:271	61-82
mmu-mir-125a	SEQ ID NO:272	6-28
mmu-mir-125b-2	SEQ ID NO:273	7-28
mmu-mir-125b-1	SEQ ID NO:274	15-36
mmu-mir-126	SEQ ID NO:275	9-29 and/or 46-66
mmu-mir-127	SEQ ID NO:276	43-64
mmu-mir-128a	SEQ ID NO:277	44-65
mmu-mir-128b	SEQ ID NO:278	48-69
mmu-mir-129-1	SEQ ID NO:279	6-27
mmu-mir-129-2	SEQ ID NO:280	15-36
mmu-mir-130a	SEQ ID NO:281	42-61
mmu-mir-130b	SEQ ID NO:282	51-72
mmu-mir-132	SEQ ID NO:283	42-63
mmu-mir-133a-1	SEQ ID NO:284	44-65
mmu-mir-133a-2	SEQ ID NO:285	60-81

miRNA name	Precursor	Processed Sequence Relative to Precursor
mmu-mir-133b	SEQ ID NO:286	67-87
mmu-mir-134	SEQ ID NO:287	7-27
mmu-mir-135a-1	SEQ ID NO:288	17-39
mmu-mir-135b	SEQ ID NO:289	16-37
mmu-mir-135a-2	SEQ ID NO:290	23-45
mmu-mir-136	SEQ ID NO:291	5-27
mmu-mir-137	SEQ ID NO:292	46-67
mmu-mir-138-2	SEQ ID NO:293	2-18
mmu-mir-138-1	SEQ ID NO:294	23-39
mmu-mir-139	SEQ ID NO:295	7-24
mmu-mir-140	SEQ ID NO:296	7-27
mmu-mir-141	SEQ ID NO:297	49-69
mmu-mir-142	SEQ ID NO:298	4-23 and/or 40-61
mmu-mir-143	SEQ ID NO:299	40-61
mmu-mir-144	SEQ ID NO:300	43-64
mmu-mir-145	SEQ ID NO:301	7-30
mmu-mir-146	SEQ ID NO:302	6-27
mmu-mir-148a	SEQ ID NO:303	61-82
mmu-mir-149	SEQ ID NO:304	4-25
mmu-mir-150	SEQ ID NO:305	6-27
mmu-mir-151	SEQ ID NO:306	43-63
mmu-mir-152	SEQ ID NO:307	47-67
mmu-mir-153	SEQ ID NO:308	44-63
mmu-mir-154	SEQ ID NO:309	6-27
mmu-mir-155	SEQ ID NO:310	4-25
mmu-mir-181a	SEQ ID NO:311	7-29
mmu-mir-181b-1	SEQ ID NO:312	12-35
mmu-mir-181c	SEQ ID NO:313	17-38
mmu-mir-181b-2	SEQ ID NO:314	16-39
mmu-mir-182	SEQ ID NO:315	7-28
mmu-mir-183	SEQ ID NO:316	6-28
mmu-mir-184	SEQ ID NO:317	45-66
mmu-mir-185	SEQ ID NO:318	7-24
mmu-mir-186	SEQ ID NO:319	7-29
mmu-mir-187	SEQ ID NO:320	40-61
mmu-mir-188	SEQ ID NO:321	6-27
mmu-mir-190	SEQ ID NO:322	6-27
mmu-mir-191	SEQ ID NO:323	7-28
mmu-mir-192	SEQ ID NO:324	14-31
mmu-mir-193	SEQ ID NO:325	41-61
mmu-mir-194-1	SEQ ID NO:326	7-28
mmu-mir-194-2	SEQ ID NO:327	16-37
mmu-mir-195	SEQ ID NO:328	1-21
mmu-mir-196-1	SEQ ID NO:329	24-44
mmu-mir-196-2	SEQ ID NO:330	16-36
mmu-mir-199a-1	SEQ ID NO:331	6-28 and/or 45-66

miRNA name	Precursor	Processed Sequence Relative to Precursor
mmu-mir-199a-2	SEQ ID NO:332	31-53 and/or 69-90
mmu-mir-199b	SEQ ID NO:333	26-48
mmu-mir-200b	SEQ ID NO:334	45-67
mmu-mir-200a	SEQ ID NO:335	54-75
mmu-mir-200c	SEQ ID NO:336	46-67
mmu-mir-201	SEQ ID NO:337	6-26
mmu-mir-202	SEQ ID NO:338	45-66
mmu-mir-203	SEQ ID NO:339	49-69
mmu-mir-204	SEQ ID NO:340	6-28
mmu-mir-205	SEQ ID NO:341	7-28
mmu-mir-206	SEQ ID NO:342	46-67
mmu-mir-207	SEQ ID NO:343	52-74
mmu-mir-208	SEQ ID NO:344	50-71
mmu-mir-210	SEQ ID NO:345	66-86
mmu-mir-211	SEQ ID NO:346	26-47
mmu-mir-212	SEQ ID NO:347	56-76
mmu-mir-213	SEQ ID NO:348	14-36 and/or 54-75
mmu-mir-214	SEQ ID NO:349	71-91
mmu-mir-215	SEQ ID NO:350	30-50
mmu-mir-216	SEQ ID NO:351	7-27
mmu-mir-217	SEQ ID NO:352	34-57
mmu-mir-218-2	SEQ ID NO:353	25-45
mmu-mir-219-1	SEQ ID NO:354	21-41
mmu-mir-219-2	SEQ ID NO:355	19-39
mmu-mir-221	SEQ ID NO:356	60-81
mmu-mir-222	SEQ ID NO:357	49-71
mmu-mir-223	SEQ ID NO:358	68-88
mmu-mir-224	SEQ ID NO:359	8-30
mu-miR-290	SEQ ID NO:360	15-37
mmu-mir-291	SEQ ID NO:361	14-35 and/or 50-72
mmu-mir-292	SEQ ID NO:362	12-33 and/or 51-73
mmu-mir-293	SEQ ID NO:363	48-69
mmu-mir-294	SEQ ID NO:364	51-72
mmu-mir-295	SEQ ID NO:365	43-65
mmu-mir-296	SEQ ID NO:366	13-33
mmu-mir-297-1	SEQ ID NO:367	15-35
mmu-mir-297-2	SEQ ID NO:368	36-56
mmu-mir-298	SEQ ID NO:369	11-32
mmu-mir-299	SEQ ID NO:370	7-28
mmu-mir-300	SEQ ID NO:371	51-72
mmu-mir-301	SEQ ID NO:372	51-73
mmu-mir-302	SEQ ID NO:373	44-66
mmu-mir-320	SEQ ID NO:374	48-70
mmu-mir-321	SEQ ID NO:375	10-30
mmu-mir-323	SEQ ID NO:376	50-71
mmu-mir-324	SEQ ID NO:377	18-40 and/or 53-74

miRNA name	Precursor	Processed Sequence Relative to Precursor
mmu-mir-325	SEQ ID NO:378	16-38
mmu-mir-326	SEQ ID NO:379	60-80
mmu-mir-328	SEQ ID NO:380	61-82
mmu-mir-329	SEQ ID NO:381	61-82
mmu-mir-330	SEQ ID NO:382	61-83
mmu-mir-331	SEQ ID NO:383	61-81
mmu-mir-337	SEQ ID NO:384	61-83
mmu-mir-338	SEQ ID NO:385	61-83
mmu-mir-339	SEQ ID NO:386	16-36
mmu-mir-340	SEQ ID NO:387	61-83
mmu-mir-341	SEQ ID NO:388	61-81
mmu-mir-342	SEQ ID NO:389	61-84
mmu-mir-344	SEQ ID NO:390	61-83
mmu-mir-345	SEQ ID NO:391	16-36
mmu-mir-346	SEQ ID NO:392	16-38
mmu-mir-350	SEQ ID NO:393	61-84
mmu-mir-351	SEQ ID NO:583	16-39
mmu-mir-370	SEQ ID NO:584	48-70
mmu-mir-376a	SEQ ID NO:585	44-64
mmu-mir-376b	SEQ ID NO:586	51-72
mmu-mir-380	SEQ ID NO:587	40-61
mmu-mir-409	SEQ ID NO:588	47-69
mmu-mir-410	SEQ ID NO:589	50-71
mmu-mir-411	SEQ ID NO:590	56-78
mmu-mir-412	SEQ ID NO:591	50-72
mmu-mir-425	SEQ ID NO:695	54-74
mmu-mir-429	SEQ ID NO:696	51-72
mmu-mir-448	SEQ ID NO:697	72-93
mmu-mir-449	SEQ ID NO:698	16-37
mmu-mir-450	SEQ ID NO:699	17-38
mmu-mir-451	SEQ ID NO:786	17-38
mmu-mir-452	SEQ ID NO:787	17-38
mmu-mir-463	SEQ ID NO:788	4-24
mmu-mir-464	SEQ ID NO:789	47-69
mmu-mir-465	SEQ ID NO:790	5-27
mmu-mir-466	SEQ ID NO:791	51-73
mmu-mir-467	SEQ ID NO:792	50-71
mmu-mir-468	SEQ ID NO:793	53-75
mmu-mir-469	SEQ ID NO:794	6-31
mmu-mir-470	SEQ ID NO:795	9-29
mmu-mir-471	SEQ ID NO:796	7-29
mmu-mir-483	SEQ ID NO:797	45-67
mmu-mir-484	SEQ ID NO:798	2-23
mmu-mir-485	SEQ ID NO:799	9-30
mmu-mir-486	SEQ ID NO:800	4-25

Table 3
Rat miRNA Sequences

miRNA name	Precursor	Processed Sequence Relative to Precursor
rno-let-7d	SEQ ID NO:394	14-34 and/or 68-89
rno-mir-7-1	SEQ ID NO:395	19-39 and/or 61-82
rno-let-7a-1	SEQ ID NO:396	13-34
rno-let-7a-2	SEQ ID NO:397	17-38
rno-let-7b	SEQ ID NO:398	7-28
rno-let-7c-1	SEQ ID NO:399	16-37
rno-let-7c-2	SEQ ID NO:400	14-35
rno-let-7e	SEQ ID NO:401	15-35
rno-let-7f-1	SEQ ID NO:402	8-29
rno-let-7f-2	SEQ ID NO:403	8-29
rno-let-7i	SEQ ID NO:404	6-24
rno-mir-7-2	SEQ ID NO:405	19-39
rno-mir-7b	SEQ ID NO:406	29-49
rno-mir-9-1	SEQ ID NO:407	16-38
rno-mir-9-3	SEQ ID NO:408	16-38
rno-mir-9-2	SEQ ID NO:409	16-38
rno-mir-10a	SEQ ID NO:410	22-44
rno-mir-10b	SEQ ID NO:411	26-47
rno-mir-15b	SEQ ID NO:412	20-41
rno-mir-16	SEQ ID NO:413	17-38
rno-mir-17	SEQ ID NO:414	14-37
rno-mir-18	SEQ ID NO:415	17-38
rno-mir-19b-1	SEQ ID NO:416	54-76
rno-mir-19b-2	SEQ ID NO:417	62-84
rno-mir-19a	SEQ ID NO:418	49-71
rno-mir-20	SEQ ID NO:419	16-38 and/or 52-72
rno-mir-21	SEQ ID NO:420	18-39
rno-mir-22	SEQ ID NO:421	57-78
rno-mir-23a	SEQ ID NO:422	46-66
rno-mir-23b	SEQ ID NO:423	58-80
rno-mir-24-1	SEQ ID NO:424	44-65
rno-mir-24-2	SEQ ID NO:425	61-82
rno-mir-25	SEQ ID NO:426	52-73
rno-mir-26a	SEQ ID NO:427	16-37
rno-mir-26b	SEQ ID NO:428	15-36
rno-mir-27b	SEQ ID NO:429	61-80
rno-mir-27a	SEQ ID NO:430	56-76
rno-mir-28	SEQ ID NO:431	14-35
rno-mir-29b-2	SEQ ID NO:432	52-73
rno-mir-29a	SEQ ID NO:433	53-74
rno-mir-29b-1	SEQ ID NO:434	51-72
rno-mir-29c	SEQ ID NO:435	54-75
rno-mir-30c-1	SEQ ID NO:436	17-39

miRNA name	Precursor	Processed Sequence Relative to Precursor
rno-mir-30e	SEQ ID NO:437	2-21
rno-mir-30b	SEQ ID NO:438	16-36
rno-mir-30d	SEQ ID NO:439	12-33
rno-mir-30a	SEQ ID NO:440	47-68
rno-mir-30c-2	SEQ ID NO:441	14-36
rno-mir-31	SEQ ID NO:442	28-49
rno-mir-32	SEQ ID NO:443	6-26
rno-mir-33	SEQ ID NO:444	6-24
rno-mir-34b	SEQ ID NO:445	13-35
rno-mir-34c	SEQ ID NO:446	13-35
rno-mir-34a	SEQ ID NO:447	20-42
rno-mir-92-1	SEQ ID NO:448	48-68
rno-mir-92-2	SEQ ID NO:449	55-75
rno-mir-93	SEQ ID NO:450	15-37
rno-mir-96	SEQ ID NO:451	24-46
rno-mir-98	SEQ ID NO:452	2-23
rno-mir-99a	SEQ ID NO:453	13-34
rno-mir-99b	SEQ ID NO:454	7-28
rno-mir-100	SEQ ID NO:455	13-34
rno-mir-101b	SEQ ID NO:456	61-82
rno-mir-101	SEQ ID NO:457	47-68
rno-mir-103-2	SEQ ID NO:458	52-74
rno-mir-103-1	SEQ ID NO:459	52-74
rno-mir-106b	SEQ ID NO:460	12-32
rno-mir-107	SEQ ID NO:461	52-74
rno-mir-122a	SEQ ID NO:462	15-37
rno-mir-124a-3	SEQ ID NO:463	52-73
rno-mir-124a-1	SEQ ID NO:464	52-73
rno-mir-124a-2	SEQ ID NO:465	61-82
rno-mir-125a	SEQ ID NO:466	15-37
rno-mir-125b-1	SEQ ID NO:467	15-36
rno-mir-125b-2	SEQ ID NO:468	17-38
rno-mir-126	SEQ ID NO:469	9-29 and/or 46-66
rno-mir-127	SEQ ID NO:470	57-78
rno-mir-128a	SEQ ID NO:471	50-71
rno-mir-128b	SEQ ID NO:472	52-73
rno-mir-129-2	SEQ ID NO:473	19-40 and/or 61-82
rno-mir-129-1	SEQ ID NO:474	6-27
rno-mir-130a	SEQ ID NO:475	55-74
rno-mir-130b	SEQ ID NO:476	51-72
rno-mir-132	SEQ ID NO:477	59-80
rno-mir-133a	SEQ ID NO:478	53-74
rno-mir-134	SEQ ID NO:479	8-28
rno-mir-135b	SEQ ID NO:480	16-37
rno-mir-135a	SEQ ID NO:481	23-45
rno-mir-136	SEQ ID NO:482	15-37

miRNA name	Precursor	Processed Sequence Relative to Precursor
rno-mir-137	SEQ ID NO:483	60-81
rno-mir-138-2	SEQ ID NO:484	9-25
rno-mir-138-1	SEQ ID NO:485	23-39
rno-mir-139	SEQ ID NO:486	7-24
rno-mir-140	SEQ ID NO:487	23-43 and/or 61-84
rno-mir-141	SEQ ID NO:488	59-79
rno-mir-142	SEQ ID NO:489	16-35 and/or 52-74
rno-mir-143	SEQ ID NO:490	60-81
rno-mir-144	SEQ ID NO:491	50-71
rno-mir-145	SEQ ID NO:492	16-39
rno-mir-146	SEQ ID NO:493	17-38
rno-mir-148b	SEQ ID NO:494	61-82
rno-mir-150	SEQ ID NO:495	16-37
rno-mir-151	SEQ ID NO:496	16-37 and/or 50-71
rno-mir-152	SEQ ID NO:497	53-73
rno-mir-153	SEQ ID NO:498	53-72
rno-mir-154	SEQ ID NO:499	15-36
rno-mir-181c	SEQ ID NO:500	24-45
rno-mir-181a	SEQ ID NO:501	39-61
rno-mir-181b-1	SEQ ID NO:502	36-59
rno-mir-181b-2	SEQ ID NO:503	15-38
rno-mir-183	SEQ ID NO:504	27-49
rno-mir-184	SEQ ID NO:505	47-68
rno-mir-185	SEQ ID NO:506	14-31
rno-mir-186	SEQ ID NO:507	15-37
rno-mir-187	SEQ ID NO:508	66-86
rno-mir-190	SEQ ID NO:509	15-36
rno-mir-191	SEQ ID NO:510	15-36
rno-mir-192	SEQ ID NO:511	24-44
rno-mir-193	SEQ ID NO:512	54-74
rno-mir-194-1	SEQ ID NO:513	15-36
rno-mir-194-2	SEQ ID NO:514	15-36
rno-mir-195	SEQ ID NO:515	15-35
rno-mir-196	SEQ ID NO:516	25-45
rno-mir-199a	SEQ ID NO:517	31-53
rno-mir-200c	SEQ ID NO:518	46-67
rno-mir-200a	SEQ ID NO:519	54-75
rno-mir-200b	SEQ ID NO:520	54-77
rno-mir-203	SEQ ID NO:521	52-73
rno-mir-204	SEQ ID NO:522	33-54
rno-mir-205	SEQ ID NO:523	33-54
rno-mir-206	SEQ ID NO:524	51-72
rno-mir-208	SEQ ID NO:525	50-71
rno-mir-210	SEQ ID NO:526	66-86
rno-mir-211	SEQ ID NO:527	26-47
rno-mir-212	SEQ ID NO:528	72-92

miRNA name	Precursor	Processed Sequence Relative to Precursor
rno-mir-213	SEQ ID NO:529	55-76
rno-mir-214	SEQ ID NO:530	71-91
rno-mir-216	SEQ ID NO:531	19-39
rno-mir-217	SEQ ID NO:532	32-55
rno-mir-218-2	SEQ ID NO:533	25-45
rno-mir-218-1	SEQ ID NO:534	25-45
rno-mir-219-1	SEQ ID NO:535	21-41
rno-mir-219-2	SEQ ID NO:536	19-39
rno-mir-221	SEQ ID NO:537	65-87
rno-mir-222	SEQ ID NO:538	62-85
rno-mir-223	SEQ ID NO:539	68-88
rno-mir-290	SEQ ID NO:540	14-36
rno-mir-291	SEQ ID NO:541	14-35 and/or 50-72
rno-mir-292	SEQ ID NO:542	12-33 and/or 51-73
rno-mir-296	SEQ ID NO:543	13-33
rno-mir-297	SEQ ID NO:544	26-48
rno-mir-298	SEQ ID NO:545	11-32
rno-mir-299	SEQ ID NO:546	7-28
rno-mir-300	SEQ ID NO:547	51-72
rno-mir-301	SEQ ID NO:548	61-85
rno-mir-320	SEQ ID NO:549	48-70
rno-mir-321	SEQ ID NO:550	10-30
rno-mir-322	SEQ ID NO:551	61-80
rno-mir-323	SEQ ID NO:552	50-71
rno-mir-324	SEQ ID NO:553	16-38 and/or 51-72
rno-mir-325	SEQ ID NO:554	16-38
rno-mir-326	SEQ ID NO:555	60-80
rno-mir-328	SEQ ID NO:556	48-69
rno-mir-329	SEQ ID NO:557	61-82
rno-mir-330	SEQ ID NO:558	60-82
rno-mir-331	SEQ ID NO:559	61-81
rno-mir-333	SEQ ID NO:560	16-35
rno-mir-336	SEQ ID NO:561	16-36
rno-mir-337	SEQ ID NO:562	60-82
rno-mir-338	SEQ ID NO:563	41-63
rno-mir-339	SEQ ID NO:564	16-36
rno-mir-341	SEQ ID NO:565	61-81
rno-mir-342	SEQ ID NO:566	61-84
rno-mir-344	SEQ ID NO:567	61-83
rno-mir-345	SEQ ID NO:568	16-36
rno-mir-346	SEQ ID NO:569	16-38
rno-mir-349	SEQ ID NO:570	61-82
rno-mir-350	SEQ ID NO:571	61-84
rno-mir-351	SEQ ID NO:572	16-39
rno-mir-352	SEQ ID NO:592	61-81
rno-mir-421	SEQ ID NO:593	10-30

miRNA name	Precursor	Processed Sequence Relative to Precursor
rno-mir-429	SEQ ID NO:700	53-74
rno-mir-448	SEQ ID NO:701	72-93
rno-mir-449	SEQ ID NO:702	16-37
rno-mir-450	SEQ ID NO:703	17-38
rno-mir-451	SEQ ID NO:801	17-38
rno-mir-483	SEQ ID NO:802	45-67

It is understood that an miRNA is derived from genomic sequences or a gene. In this respect, the term "gene" is used for simplicity to refer to the genomic sequence encoding the precursor miRNA for a given miRNA. However, embodiments of the invention may involve genomic sequences of a miRNA that are involved in its expression, such as a promoter or other regulatory sequences.

The term "recombinant" may be used and this generally refers to a molecule that has been manipulated *in vitro* or that is the replicated or expressed product of such a molecule.

The term "nucleic acid" is well known in the art. A "nucleic acid" as used herein will generally refer to a molecule (one or more strands) of DNA, RNA or a derivative or analog thereof, comprising a nucleobase. A nucleobase includes, for example, a naturally occurring purine or pyrimidine base found in DNA (*e.g.*, an adenine "A," a guanine "G," a thymine "T" or a cytosine "C") or RNA (*e.g.*, an A, a G, an uracil "U" or a C). The term "nucleic acid" encompass the terms "oligonucleotide" and "polynucleotide," each as a subgenus of the term "nucleic acid."

The term "miRNA" generally refers to a single-stranded molecule, but in specific embodiments, molecules implemented in the invention will also encompass a region or an additional strand that is partially (between 10 and 50% complementary across length of strand), substantially (greater than 50% but less than 100% complementary across length of strand) or fully complementary to another region of the same single-stranded molecule or to another nucleic acid. Thus, nucleic acids may encompass a molecule that comprises one or more complementary or self-complementary strand(s) or "complement(s)" of a particular sequence comprising a molecule. For example, precursor miRNA may have a self-complementary region, which is up to 100% complementary.

As used herein, "hybridization", "hybridizes" or "capable of hybridizing" is understood to mean the forming of a double or triple stranded molecule or a molecule with partial double or triple stranded nature. The term “anneal” as used herein is synonymous with “hybridize.” The term "hybridization", "hybridize(s)" or "capable of hybridizing" encompasses the terms "stringent condition(s)" or "high stringency" and the terms "low stringency" or "low stringency condition(s)."

Synthetic nucleic acids of the invention will comprise, in some embodiments the miRNA sequence of any miRNA described in SEQ ID NOs:1-805, and/or any sequence with the complement thereof. It is contemplated that nucleic acids sequences of the invention can have, have at least, or have at most 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150 contiguous nucleotides from SEQ ID NOs:1-805 (or any ranger derivable therein), or be a complement thereof. In other embodiments, nucleic acids are, are at least, or are at most 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% identical or complementary to the miRNA sequence of SEQ ID NOs:1-805 or to the entire sequence of any of SEQ ID NOs:1-805, or any combination or range derivable therein.

Moreover, sequences are provided in the appendix. The appendix provides a list of 1) miRNAs that were screeened, any one of which can be screened for using any array or method of the present invention; 2) the names of the probe used to screen for that miRNA; and, 3) the sequence of the named probe. It is clear that a particular probe can be used for identifying the level of expression of one or more target miRNAs, or set of target miRNAs (sets of targeted miRNAs may include completely unrelated RNAs, inadditions to sets that are either related or in the same gene family).

It is contemplated that any of these sequences in the appendix can be used in embodiments of the invention.

1. Nucleobases

As used herein a "nucleobase" refers to a heterocyclic base, such as for example a naturally occurring nucleobase (*i.e.*, an A, T, G, C or U) found in at least one naturally occurring nucleic acid (*i.e.*, DNA and RNA), and naturally or non-naturally occurring derivative(s) and analogs of such a nucleobase. A nucleobase generally can form one or more hydrogen bonds ("anneal" or "hybridize") with at least one naturally occurring nucleobase in manner that may substitute for naturally occurring nucleobase pairing (*e.g.*, the hydrogen bonding between A and T, G and C, and A and U).

"Purine" and/or "pyrimidine" nucleobase(s) encompass naturally occurring purine and/or pyrimidine nucleobases and also derivative(s) and analog(s) thereof, including but not limited to, those a purine or pyrimidine substituted by one or more of an alkyl, caboxyalkyl, amino, hydroxyl, halogen (*i.e.*, fluoro, chloro, bromo, or iodo), thiol or alkylthiol moeity. Preferred alkyl (*e.g.*, alkyl, caboxyalkyl, etc.) moieties comprise of from about 1, about 2, about 3, about 4, about 5, to about 6 carbon atoms. Other non-limiting examples of a purine or pyrimidine include a deazapurine, a 2,6-diaminopurine, a 5-fluorouracil, a xanthine, a hypoxanthine, a 8-bromoguanine, a 8-chloroguanine, a bromothymine, a 8-aminoguanine, a 8-hydroxyguanine, a 8-methylguanine, a 8-thioguanine, an azaguanine, a 2-aminopurine, a 5-ethylcytosine, a 5-methylcyosine, a 5-bromouracil, a 5-ethyluracil, a 5-iodouracil, a 5-chlorouracil, a 5-propyluracil, a thiouracil, a 2-methyladenine, a methylthioadenine, a N,N-diemethyladenine, an azaadenines, a 8-bromoadenine, a 8-hydroxyadenine, a 6-hydroxyaminopurine, a 6-thiopurine, a 4-(6-aminohexyl/cytosine), and the like. Other examples are well known to those of skill in the art.

A nucleobase may be comprised in a nucleoside or nucleotide, using any chemical or natural synthesis method described herein or known to one of ordinary skill in the art. Such nucleobase may be labeled or it may be part of a molecule that is labeled and contains the nucleobase.

2. Nucleosides

As used herein, a "nucleoside" refers to an individual chemical unit comprising a nucleobase covalently attached to a nucleobase linker moiety. A non-limiting example of a "nucleobase linker moiety" is a sugar comprising 5-carbon atoms (*i.e.*, a "5-carbon sugar"), including but not limited to a deoxyribose, a ribose, an arabinose, or a derivative or an analog of a 5-carbon sugar. Non-limiting examples of a derivative or an analog of a 5-carbon sugar include a 2'-fluoro-2'-deoxyribose or a carbocyclic sugar where a carbon is substituted for an oxygen atom in the sugar ring.

Different types of covalent attachment(s) of a nucleobase to a nucleobase linker moiety are known in the art. By way of non-limiting example, a nucleoside comprising a purine (*i.e.*, A or G) or a 7-deazapurine nucleobase typically covalently attaches the 9 position of a purine or a 7-deazapurine to the 1'-position of a 5-carbon sugar. In another non-limiting example, a nucleoside comprising a pyrimidine nucleobase (*i.e.*, C, T or U) typically covalently attaches a 1 position of a pyrimidine to a 1'-position of a 5-carbon sugar (Kornberg and Baker, 1992).

3. Nucleotides

As used herein, a "nucleotide" refers to a nucleoside further comprising a "backbone moiety". A backbone moiety generally covalently attaches a nucleotide to another molecule comprising a nucleotide, or to another nucleotide to form a nucleic acid. The "backbone moiety" in naturally occurring nucleotides typically comprises a phosphorus moiety, which is covalently attached to a 5-carbon sugar. The attachment of the backbone moiety typically occurs at either the 3′- or 5′-position of the 5-carbon sugar. However, other types of attachments are known in the art, particularly when a nucleotide comprises derivatives or analogs of a naturally occurring 5-carbon sugar or phosphorus moiety.

4. Nucleic Acid Analogs

A nucleic acid may comprise, or be composed entirely of, a derivative or analog of a nucleobase, a nucleobase linker moiety and/or backbone moiety that may be present in a naturally occurring nucleic acid. RNA with nucleic acid analogs may also be labeled according to methods of the invention. As used herein a "derivative" refers to a chemically modified or altered form of a naturally occurring molecule, while the terms "mimic" or "analog" refer to a molecule that may or may not structurally resemble a naturally occurring molecule or moiety, but possesses similar functions. As used herein, a "moiety" generally refers to a smaller chemical or molecular component of a larger chemical or molecular structure. Nucleobase, nucleoside and nucleotide analogs or derivatives are well known in the art, and have been described (see for example, Scheit, 1980).

Additional non-limiting examples of nucleosides, nucleotides or nucleic acids comprising 5-carbon sugar and/or backbone moiety derivatives or analogs, include those in: U.S. Patent No. 5,681,947, which describes oligonucleotides comprising purine derivatives that form triple helixes with and/or prevent expression of dsDNA; U.S. Patents 5,652,099 and 5,763,167, which describe nucleic acids incorporating fluorescent analogs of nucleosides found in DNA or RNA, particularly for use as fluorescent nucleic acids probes; U.S. Patent 5,614,617, which describes oligonucleotide analogs with substitutions on pyrimidine rings that possess enhanced nuclease stability; U.S. Patents 5,670,663, 5,872,232 and 5,859,221, which describe oligonucleotide analogs with modified 5-carbon sugars (*i.e.,* modified 2'-deoxyfuranosyl moieties) used in nucleic acid detection; U.S. Patent 5,446,137, which describes oligonucleotides comprising at least one 5-carbon sugar moiety substituted at the 4' position with a substituent other than hydrogen that can be used in hybridization assays; U.S. Patent 5,886,165, which describes oligonucleotides with both deoxyribonucleotides with 3′-5′ internucleotide linkages and ribonucleotides with 2′-5′ internucleotide linkages; U.S. Patent 5,714,606, which describes a modified internucleotide linkage wherein a 3′-position oxygen of the internucleotide linkage is replaced by a carbon to enhance the nuclease resistance of nucleic acids; U.S. Patent 5,672,697, which describes oligonucleotides containing one or more 5' methylene phosphonate internucleotide linkages that enhance nuclease resistance; U.S. Patents 5,466,786 and 5,792,847, which describe the linkage of a substituent moiety which may comprise a drug or label to the 2' carbon of an oligonucleotide to provide enhanced nuclease stability and ability to deliver drugs or detection moieties; U.S. Patent 5,223,618, which describes oligonucleotide analogs with a 2 or 3 carbon backbone linkage attaching the 4' position and 3' position of adjacent 5-carbon sugar moiety to enhanced cellular uptake, resistance to nucleases and hybridization to target RNA; U.S. Patent 5,470,967, which describes oligonucleotides comprising at least one sulfamate or sulfamide internucleotide linkage that are useful as nucleic acid hybridization probe; U.S. Patents 5,378,825, 5,777,092, 5,623,070, 5,610,289 and 5,602,240, which describe oligonucleotides with three or four atom linker moiety replacing phosphodiester backbone moiety used for improved nuclease resistance, cellular uptake and regulating RNA expression; U.S. Patent 5,858,988, which describes hydrophobic carrier agent attached to the 2'-O position of oligonucleotides to enhanced their membrane permeability and stability; U.S. Patent 5,214,136, which describes oligonucleotides conjugated to anthraquinone at the 5′ terminus that possess enhanced hybridization to DNA or RNA; enhanced stability to nucleases; U.S. Patent 5,700,922, which describes PNA-DNA-PNA chimeras wherein the DNA comprises 2'-deoxy-erythro-pentofuranosyl nucleotides for enhanced nuclease resistance, binding affinity, and ability to activate RNase H; and U.S. Patent 5,708,154, which describes RNA linked to a DNA to form a DNA-RNA hybrid; U.S. Patent 5,728,525, which describes the labeling of nucleoside analogs with a universal fluorescent label.

Additional teachings for nucleoside analogs and nucleic acid analogs are U.S. Patent 5,728,525, which describes nucleoside analogs that are end-labeled; U.S. Patent 5,637,683, 6,251,666 (L-nucleotide substitutions), and 5,480,980 (7-deaza-2'deoxyguanosine nucleotides and nucleic acid analogs thereof).

The use of other analogs is specifically contemplated for use in the context of the present invention. Such analogs may be used in synthetic nucleic acid molecules of the invention, both throughout the molecule or at selected nucleotides. They include, but are not limited to, 1) ribose modifications (such as 2'F, 2' NH_2, 2'N_3, 4'thio, or 2' O-CH_3) and 2) phosphate modifications (such as those found in phosphorothioates, methyl phosphonates, and phosphoroborates). Such analogs have been created to confer stability on RNAs by reducing or eliminating their capacity to be cleaved by ribonucleases. When these nucleotide analogs are present in RNAs, they can have profoundly positive effects on the stability of the RNAs in animals. It is contemplated that the use of nucleotide analogs can be used alone or in conjunction with any of the design modifications of a synthetic miRNA for any nucleic acid of the invention.

5. Modified Nucleotides

Both synthetic miRNAs and miRNA inhibitors of the invention specifically contemplate the use of nucleotides that are modified to enhance their activities. Such nucleotides include those that are at the 5′ or 3′ terminus of the RNA as well as those that are internal within the molecule. Modified nucleotides used in the complementary strands of synthetic miRNAs either block the 5'OH or phosphate of the RNA or introduce internal sugar modifications that enhance uptake of the active strand of the synthetic miRNA. Modifications for the miRNA inhibitors include internal sugar modifications that enhance hybridization as well as stabilize the molecules in cells and terminal modifications that further stabilize the nucleic acids in cells. Further contemplated are modifications that can be detected by microscopy or other methods to identify cells that contain the synthetic miRNAs or miRNA inhibitors.

B. Preparation of Nucleic Acids

A nucleic acid may be made by any technique known to one of ordinary skill in the art, such as for example, chemical synthesis, enzymatic production or biological production. Though synthetic miRNAs according to the invention could be produced using recombinant methods, it is preferred to produce synthetic miRNAs by chemical synthesis or enzymatic production. Likewise, miRNA inhibitors are preferentially produced by chemical synthesis or enzymatic production. Non-synthetic miRNAs can be produced by a number of methods, including methods involving recombinant DNA technology.

Nucleic acid synthesis is performed according to standard methods. See, for example, Itakura and Riggs (1980). Additionally, U.S. Patent 4,704,362, U.S. Patent 5,221,619, and U.S. Patent 5,583,013 each describe various methods of preparing synthetic nucleic acids. Non-limiting examples of a synthetic nucleic acid (*e.g.*, a synthetic oligonucleotide), include a nucleic acid made by *in vitro* chemically synthesis using phosphotriester, phosphite or phosphoramidite chemistry and solid phase techniques such as described in EP 266,032 or via deoxynucleoside H-phosphonate intermediates as described by Froehler *et al.*, 1986 and U.S. Patent Serial No. 5,705,629. In the methods of the present invention, one or more oligonucleotide may be used. Various different mechanisms of oligonucleotide synthesis have been disclosed in for example, U.S. Patents. 4,659,774, 4,816,571, 5,141,813, 5,264,566, 4,959,463, 5,428,148, 5,554,744, 5,574,146, 5,602,244.

A non-limiting example of an enzymatically produced nucleic acid include one produced by enzymes in amplification reactions such as PCR™ (see for example, U.S. Patent 4,683,202 and U.S. Patent 4,682,195 or the synthesis of an oligonucleotide described in U.S. Patent No. 5,645,897.

Oligonucleotide synthesis is well known to those of skill in the art. Various different mechanisms of oligonucleotide synthesis have been disclosed in for example, U.S. Patents 4,659,774, 4,816,571, 5,141,813, 5,264,566, 4,959,463, 5,428,148, 5,554,744, 5,574,146, 5,602,244.

Basically, chemical synthesis can be achieved by the diester method, the triester method polynucleotides phosphorylase method and by solid-phase chemistry. These methods are discussed in further detail below.

Diester method. The diester method was the first to be developed to a usable state, primarily by Khorana and co-workers. (Khorana, 1979). The basic step is the joining of two suitably protected deoxynucleotides to form a dideoxynucleotide containing a phosphodiester bond. The diester method is well established and has been used to synthesize DNA molecules (Khorana, 1979).

Triester method. The main difference between the diester and triester methods is the presence in the latter of an extra protecting group on the phosphate atoms of the reactants and products (Itakura *et al.*, 1975). The phosphate protecting group is usually a chlorophenyl group, which renders the nucleotides and polynucleotide intermediates soluble in organic solvents. Therefore purification's are done in chloroform solutions. Other improvements in the method include (i) the block coupling of trimers and larger oligomers, (ii) the extensive use of high-performance liquid chromatography for the purification of both intermediate and final products, and (iii) solid-phase synthesis.

Polynucleotide phosphorylase method. This is an enzymatic method of DNA synthesis that can be used to synthesize many useful oligonucleotides (Gillam *et* *al.*, 1978; Gillam *et al.*, 1979). Under controlled conditions, polynucleotide phosphorylase adds predominantly a single nucleotide to a short oligonucleotide. Chromatographic purification allows the desired single adduct to be obtained. At least a trimer is required to start the procedure, and this primer must be obtained by some other method. The polynucleotide phosphorylase method works and has the advantage that the procedures involved are familiar to most biochemists.

Solid-phase methods. Drawing on the technology developed for the solid-phase synthesis of polypeptides, it has been possible to attach the initial nucleotide to solid support material and proceed with the stepwise addition of nucleotides. All mixing and washing steps are simplified, and the procedure becomes amenable to automation. These syntheses are now routinely carried out using automatic nucleic acid synthesizers.

Phosphoramidite chemistry (Beaucage and Lyer, 1992) has become by far the most widely used coupling chemistry for the synthesis of oligonucleotides. As is well known to those skilled in the art, phosphoramidite synthesis of oligonucleotides involves activation of nucleoside phosphoramidite monomer precursors by reaction with an activating agent to form activated intermediates, followed by sequential addition of the activated intermediates to the growing oligonucleotide chain (generally anchored at one end to a suitable solid support) to form the oligonucleotide product.

Recombinant methods. Recombinant methods for producing nucleic acids in a cell are well known to those of skill in the art. These include the use of vectors, plasmids, cosmids, and other vehicles for delivery a nucleic acid to a cell, which may be the target cell or simply a host cell (to produce large quantities of the desired RNA molecule). Alternatively, such vehicles can be used in the context of a cell free system so long as the reagents for generating the RNA molecule are present. Such methods include those described in Sambrook, 2003, Sambrook, 2001 and Sambrook, 1989.

In certain embodiments, the present invention concerns nucleic acid molecules that are not synthetic. In some embodiments, the nucleic acid molecule has a chemical structure of a naturally occuring nucleic acid and a sequence of a naturally occuring nucleic acid, such as the exact and entire sequence of a single stranded primary miRNA (see Lee 2002), a single-stranded precursor miRNA, or a single-stranded mature miRNA. In addition to the use of recombinant technology, such non-synthetic nucleic acids may be generated chemically, such as by employing technology used for creating oligonucleotides.

C. Design of Synthetic miRNAs

Synthetic miRNAs typically comprise two strands, an active strand that is identical in sequence to the mature miRNA that is being studied and a complemenrtary strand that is at least partially complementary to the active strand. The active strand is the biologically relevant molecule and should be preferentially taken up by the complex in cells that modulates translation either through mRNA degradation or translational control. Preferential uptake of the active strand has two profound results: (1) the observed activity of the synthetic miRNA increases dramatically and (2) non-intended effects induced by uptake and activation of the complementary strand are essentially eliminated. According to the invention, several synthetic miRNA designs can be used to ensure the preferential uptake of the active strand.

5′ Blocking Agent. The introduction of a stable moiety other than phosphate or hydroxyl at the 5′ end of the complementary strand impairs its activity in the miRNA pathway. This ensures that only the active strand of the synthetic miRNA will be used to regulate translation in the cell. 5′ modifications include, but are not limited to, NH_2, biotin, an amine group, a lower alkylamine group, an acetyl group, 2'O-Me, DMTO, fluoroscein, a thiol, or acridine or any other group with this type of functionality.

Other sense strand modifications. The introduction of nucleotide modifications like 2'-OMe, NH_2, biotin, an amine group, a lower alkylamine group, an acetyl group, DMTO, fluoroscein, a thiol, or acridine or any other group with this type of functionality in the complementary strand of the synthetic miRNA can eliminate the activity of the complementary strand and enhance uptake of the active strand of the miRNA.

Base mismatches in the sense strand. As with siRNAs (Schwarz 2003), the relative stability of the 5′ and 3′ ends of the active strand of the synthetic miRNA apparently determines the uptake and activation of the active by the miRNA pathway. Destabilizing the 5′ end of the active strand of the synthetic miRNA by the strategic placement of base mismatches in the 3′ end of the complementary strand of the synthetic miRNA enhances the activity of the active strand and essentially eliminates the activity of the complementary strand.

D. Host Cells and Target Cells

The cells used to understand miRNA function may be derived from or contained in any organism (*e.g.,* plant, animal, protozoan, virus, bacterium, or fungus). The plant may be a monocot, dicot or gynmosperm; the animal may be a vertebrate or invertebrate. Preferred microbes are those used in agriculture or by industry, and those that a pathogenic for plants or animals. Fungi include organisms in both the mold and yeast morphologies. Examples of vertebrates include fish and mammals, including cattle, goat, pig, sheep, hamster, mouse, rate and human; invertebrate animals include nematodes, insects, arachnids, and other arthropods. Preferably, the cell is a vertebrate cell. More preferably, the cell is a mammalian cell.

The cells used to understand miRNA function may be from the germ line or somatic, totipotent or pluripotent, dividing or non-dividing, parenchyma or epithelium, immortalized or transformed, or the like. The cell can be a gamete or an embryo; if an embryo, it can be a single cell embryo or a constituent cell or cells from a multicellular embryo. The term "embryo" thus encompasses fetal tissue. The cell used for miRNA functional analysis may be an undifferentiated cell, such as a stem cell, or a differentiated cell, such as from a cell of an organ or tissue, including fetal tissue, or any other cell present in an organism. Cell types that are differentiated include adipocytes, fibroblasts, myocytes, cardiomyocytes, endothelium, neurons, glia, blood cells, megakaryocytes, lymphocytes, macrophages, neutrophils, eosinophils, basophils, mast cells, leukocytes, granulocytes, keratinocytes, chondrocytes, osteoblasts, osteoclasts, hepatocytes, and cells, of the endocrine or exocrine glands. Alternatively, cells may be qualified as germ cells, nurse cells, epithelial cells, endothelial cells, hormone secreting cells, contractile cells, skeletal muscle cells, cardiac muscle cells, blood cells, or cells from the bone, bone marrow, brain, breast, cervix, colon, gastrointestinal tract, heart, kidney, large intestine, liver, lung, lymph nodes, ovary, pancreas, prostate, small intestine, spine or spinal cord, spleen, stomach, testes, thymus, or uterus.

As used herein, the terms “cell,” “cell line,” and “cell culture” may be used interchangeably. All of these terms also include their progeny, which is any and all subsequent generations formed by cell division. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations. A host cell may be “transfected” or “transformed,” which refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A transformed cell includes the primary subject cell and its progeny. As used herein, the terms "engineered" and "recombinant" cells or host cells are intended to refer to a cell into which an exogenous nucleic acid sequence, such as, for example, a small, interfering RNA or a template construct encoding a reporter gene has been introduced. Therefore, recombinant cells are distinguishable from naturally occurring cells that do not contain a recombinantly introduced nucleic acid.

A tissue may comprise a host cell or cells to be transformed or contacted with a nucleic acid delivery composition and/or an additional agent. The tissue may be part or separated from an organism. In certain embodiments, a tissue and its constituent cells may comprise, but is not limited to, blood (*e.g.*, hematopoietic cells (such as human hematopoietic progenitor cells, human hematopoietic stem cells, $CD34^+$ cells $CD4^+$cells), lymphocytes and other blood lineage cells), bone marrow, brain, stem cells, blood vessel, liver, lung, bone, breast, cartilage, cervix, colon, cornea, embryonic, endometrium, endothelial, epithelial, esophagus, facia, fibroblast, follicular, ganglion cells, glial cells, goblet cells, kidney, lymph node, muscle, neuron, ovaries, pancreas, peripheral blood, prostate, skin, skin, small intestine, spleen, stomach, testes.

In certain embodiments, the host cell or tissue may be comprised in at least one organism. In certain embodiments, the organism may be, human, primate or murine. In other embodiments the organism may be any eukaryote or even a prokayrote (*e.g.,* a eubacteria, an archaea), as would be understood by one of ordinary skill in the art. One of skill in the art would further understand the conditions under which to incubate all of the above described host cells to maintain them and to permit their division to form progeny.

E. Labels and Tags

Synthetic miRNAs and miRNA inhibitors may be labeled with a radioactive, enzymatic, colorimetric, or other label or tag for detection or isolation purposes. Nucleic acids may be labeled with fluorescence in some embodiments of the invention. The fluorescent labels contemplated for use as conjugates include, but are not limited to, Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy3, Cy5, 6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, Renographin, ROX, SYPRO, TAMRA, TET, Tetramethylrhodamine, and/or Texas Red.

It is contemplated that synthetic miRNAs and miRNA inhibitors may be labeled with two different labels. Furthermore, fluorescence resonance energy transfer (FRET) may be employed in methods of the invention (*e.g.,* Klostermeier *et al.*, 2002; Emptage, 2001; Didenko, 2001).

A number of techniques for visualizing or detecting labeled nucleic acids are readily available. The reference by Stanley T. Crooke, 2000 has a discussion of such techniques (Chapter 6). Such techniques include, microscopy, arrays, Fluorometry, Light cyclers or other real time PCR™ machines, FACS analysis, scintillation counters, Phosphoimagers, Geiger counters, MRI, CAT, antibody-based detection methods (Westerns, immunofluorescence, immunohistochemistry), histochemical techniques, HPLC (Griffey *et al.*, 1997, spectroscopy, capillary gel electrophoresis (Cummins *et al.*, 1996), spectroscopy; mass spectroscopy; radiological techniques; and mass balance techniques. Alternatively, nucleic acids may be labeled or tagged to allow for their efficient isolation. In other embodiments of the invention, nucleic acids are biotinylated.

F. Delivery Methods

The present invention involves in some embodiments delivering a nucleic acid into a cell. This may be done as part of a screening method, or it may be related to a therapeutic or diagnostic application.

RNA molecules may be encoded by a nucleic acid molecule comprised in a vector. The term "vector" is used to refer to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. A nucleic acid sequence can be "exogenous," which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (*e.g.,* YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques, which are described in Sambrook *et al.*, 1989 and Ausubel *et al.,* 1996. In addition to encoding a modified polypeptide such as modified gelonin, a vector may encode non-modified polypeptide sequences such as a tag or targetting molecule. A targetting molecule is one that directs the desired nucleic acid to a particular organ, tissue, cell, or other location in a subject's body.

The term "expression vector" refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are described

There are a number of ways in which expression vectors may be introduced into cells. In certain embodiments of the invention, the expression vector comprises a virus or engineered vector derived from a viral genome. The ability of certain viruses to enter cells via receptor-mediated endocytosis, to integrate into host cell genome and express viral genes stably and efficiently have made them attractive candidates for the transfer of foreign genes into mammalian cells (Ridgeway, 1988; Nicolas and Rubenstein, 1988; Baichwal and Sugden, 1986; Temin, 1986). The first viruses used as gene vectors were DNA viruses including the papovaviruses (simian virus 40, bovine papilloma virus, and polyoma) (Ridgeway, 1988; Baichwal and Sugden, 1986) and adenoviruses (Ridgeway, 1988; Baichwal and Sugden, 1986). These have a relatively low capacity for foreign DNA sequences and have a restricted host spectrum. Furthermore, their oncogenic potential and cytopathic effects in permissive cells raise safety concerns. They can accommodate only up to 8 kb of foreign genetic material but can be readily introduced in a variety of cell lines and laboratory animals (Nicolas and Rubenstein, 1988; Temin, 1986).

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells; they can also be used as vectors. Other viral vectors may be employed as expression constructs in the present invention. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Baichwal and Sugden, 1986; Coupar *et al.,* 1988) adeno-associated virus (AAV) (Ridgeway, 1988; Baichwal and Sugden, 1986; Hermonat and Muzycska, 1984) and herpesviruses may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Baichwal and Sugden, 1986; Coupar *et al.,* 1988; Horwich *et al.,* 1990).

Other suitable methods for nucleic acid delivery to effect expression of compositions of the present invention are believed to include virtually any method by which a nucleic acid (*e.g.*, DNA, including viral and nonviral vectors) can be introduced into an organelle, a cell, a tissue or an organism, as described herein or as would be known to one of ordinary skill in the art. Such methods include, but are not limited to, direct delivery of DNA such as by injection (U.S. Patent Nos. 5,994,624, 5,981,274, 5,945,100, 5,780,448, 5,736,524, 5,702,932, 5,656,610, 5,589,466 and 5,580,859), including microinjection (Harlan and Weintraub, 1985; U.S. Patent No. 5,789,215); by electroporation (U.S. Patent No. 5,384,253); by calcium phosphate precipitation (Graham and Van Der Eb, 1973; Chen and Okayama, 1987; Rippe *et al.*, 1990); by using DEAE-dextran followed by polyethylene glycol (Gopal, 1985); by direct sonic loading (Fechheimer *et al.*, 1987); by liposome mediated transfection (Nicolau and Sene, 1982; Fraley *et al.*, 1979; Nicolau *et al.*, 1987; Wong *et al.*, 1980; Kaneda *et al.*, 1989; Kato *et al.*, 1991); by microprojectile bombardment (PCT Application Nos. WO 94/09699 and 95/06128; U.S. Patent Nos. 5,610,042; 5,322,783 5,563,055, 5,550,318, 5,538,877 and 5,538,880); by agitation with silicon carbide fibers (Kaeppler *et al.,* 1990; U.S. Patent Nos. 5,302,523 and 5,464,765); by *Agrobacterium*-mediated transformation (U.S. Patent Nos. 5,591,616 and 5,563,055); or by PEG-mediated transformation of protoplasts (Omirulleh *et al.,* 1993; U.S. Patent Nos. 4,684,611 and 4,952,500); by desiccation/inhibition-mediated DNA uptake (Potrykus *et al.,* 1985). Through the application of techniques such as these, organelle(s), cell(s), tissue(s) or organism(s) may be stably or transiently transformed.

II. Screening with Synthetic miRNA and miRNA Inhibitor Libraries

As used in the patent application, screening is a process wherein multiple miRNA-specific reagents are delivered separately into individual cell populations or animals. At one or more designated times after delivery, the cell populations or animals are assayed for one or more phenotypes. Those cells or animals that have a significantly different phenotype than cells or animals in the negative control group are classified as positives. The miRNA that was being manipulated in the sample is defined as a hit. Hits represent targets for additional research and potential therapeutic development.

In some embodiments, there is a multi-step process for screening. In certain embodiments, there are four general steps:

(1) Develop quantitative assay to monitor cellular process being studied.

Assays that measure the intensity of a cellular phenotype range from microscopic assays that monitor cell size, cell cycle status, or antibody staining to enzymatic assays that assess the turnover of a specific substrate in a cell lysate to direct measurements of biomolecules or small molecules in lysates, on cells, or in medium.

Critical to the success of a screen is creating an assay that truly measures the cellular phenotype and maximizing the signal-to-noise ratio of the assay. Maximizing signal-to-noise involves testing variables like assay time, assay components, cell type, and length of time between transfection and assay. The greater the difference in the assay results between a positive phenotype and a negative control phenotype, the greater the spread will be in the screening results and the better the opportunity will be to identify interesting genes.

(2) Optimize transfection conditions for the desired cells.

The first step in this process is identifying a transfection reagent and plating conditions that maximize the uptake of synthetic miRNAs or miRNA inhibitors while maintaining high cell viability. We find it useful to test 2-5 different transfection reagents when using cell lines or 5-10 elelctroporation conditions when using primary or suspension cells. Transfection can be optimized for the reagent or electroporation condition that worked best among the conditions tested. Screening miRNA-specific libraries requires conditions for high-throughput transfection. The inventors have developed and used a rapid process that facilitates the transfection of up to 1,000 wells in less than an hour without the need for robotics (see delivery below).

(3) Screen

Once the assay and transfection process have been developed, a library of synthetic miRNAs or miRNA inhibitors can be introduced sequentially into cells in a 24- or 96-well plate. Triplicate transfections for each reagent provide enough data for reasonable statistical analysis.

(4) Validate hits

Validating a hit involves showing that the observed phenotype is due to the miRNA being targeted. Hits are typically confirmed by delivering a dilution series of the miRNA inhibitor or synthetic miRNA that registered as a hit into the cell that was originally assayed. It has been the experience of the inventors that true hits show a dose response.

A. Synthetic miRNA and miRNA Inhibitor Library Preparation

The present invention concerns the preparation and use of synthetic miRNA and miRNA inhibitor libraries to induce changes in the activity of specific miRNAs in cells. Preparation of synthetic miRNAs and miRNA inhibitors typically involves the chemical synthesis of the active and complementary strands of the synthetic miRNA and the single-stranded miRNA inhibitor using any of the methods described in this application. If the active and complementary strands of the synthetic miRNAs are two distinct molecules, then the two strands must be hybridized prior to delivery. Hybridization can be achieved by mixing the two nucleic acids together in roughly equimolar amounts and incubating for a time and at a temperature that is appropriate for hybridization. The addition of salt (*e.g.*, NaCl or NaOAC) enhances hybridization as does the inclusion of a heat denaturation step prior to the incubation used for hybridization.

B. Delivery of Synthetic miRNAs and miRNA Inhibitors

Libraries of the invention can be used to sequentially up- or down-regulate one or more miRNAs in samples. This requires methods for introducing the synthetic miRNAs and miRNA inhibitors into cell types with associated cell assays. Lipid-based transfection is typically employed to introduce the nucleic acids inito immortalized cells and electroporation for primary cells.

Suitable methods for nucleic acid delivery according to the present invention are believed to include virtually any method by which a nucleic acid (*e.g.*, DNA, RNA, including viral and nonviral vectors) can be introduced into an organelle, a cell, a tissue or an organism, as described herein or as would be known to one of ordinary skill in the art. Such methods include, but are not limited to, direct delivery of nucleic acids such as by injection (U.S. Patents 5,994,624, 5,981,274, 5,945,100, 5,780,448, 5,736,524, 5,702,932, 5,656,610, 5,589,466 and 5,580,859), including microinjection (Harland and Weintraub, 1985; U.S. Patent 5,789,215); by electroporation (U.S. Patent No. 5,384,253); by calcium phosphate precipitation (Graham and Van Der Eb, 1973; Chen and Okayama, 1987; Rippe *et al.*, 1990); by using DEAE-dextran followed by polyethylene glycol (Gopal, 1985); by direct sonic loading (Fechheimer *et al.*, 1987); by liposome mediated transfection (Nicolau and Sene, 1982; Fraley *et al.*, 1979; Nicolau *et al.*, 1987; Wong *et al.*, 1980; Kaneda *et al.*, 1989; Kato *et al.*, 1991); by microprojectile bombardment (PCT Application Nos. WO 94/09699 and 95/06128; U.S. Patents 5,610,042; 5,322,783 5,563,055, 5,550,318, 5,538,877 and 5,538,880); by agitation with silicon carbide fibers (Kaeppler *et al.*, 1990; U.S. Patents 5,302,523 and 5,464,765); by *Agrobacterium*-mediated transformation (U.S. Patents 5,591,616 and 5,563,055); or by PEG-mediated transformation of protoplasts (Omirulleh *et al.*, 1993; U.S. Patents 4,684,611 and 4,952,500); by desiccation/inhibition-mediated DNA uptake

(Potrykus *et al.*, 1985). Through the application of techniques such as these, organelle(s), cell(s), tissue(s) or organism(s) may be stably or transiently transformed.

A variety of compounds have been attached to the ends of oligonucleotides to facilitate their transport across cell membranes. Short signal peptides found in the HIV TAT, HSV VP22, *Drosphila antennapedia*, and other proteins have been found to enable the rapid transfer of biomolecules across membranes (reviewed by Schwarze 2000). These signal peptides, referred to as Protein Transduction Domains (PTDs), have been attached to oligonucleotides to facilitate their delivery into cultured cells. Cholesterols have been conjugated to oligonucleotides to improve their uptake into cells in animals (MacKellar 1992). The terminal cholesterol groups apparently interact with receptors or lipids on the surfaces of cells and facilitate the internalization of the modified oligonucleotides. Likewise, poly-l-lysine has been conjugated to oligonucleotides to decrease the net negative charge and improve uptake into cells (Leonetti 1990).

A variety of compounds have been developed that complex with nucleic acids, deliver them to surfaces of cells, and facilitate their uptake in and release from endosomes. Among these are: (1) a variety of lipids such as DOTAP (or other cationic lipid), DDAB, DHDEAB, and DOPE and (2) non-lipid-based polymers like polyethylenimine, polyamidoamine, and dendrimers of these and other polymers. In certain of these embodiments a combination of lipids is employed such as DOTAP and cholesterol or a cholesterol derivative (U.S. Patent 6,770,291). Several of these reagents have been shown to facilitate nucleic acid uptake in animals.

The cellular components involved in the miRNA pathway are becoming known. Proteins that stabilize and/or transport miRNAs within cells might enhance the stability and activity of miRNAs because they should protect and guide the bound miRNAs once they are in cells. Mixtures of miRNA-transporter proteins and miRNAs could enhance the efficacy of miRNA-based therapeutics.

RNAs are hydrophilic molecules by virtue of their anionic phosphate and sugar backbone. Although the nucleobases are hydrophobic, hydrophilicity dominates owing to the extensive hydrogen bonding resulting from the phosphate and sugar residues. The hydrophilic character and anionic backbone reduces cellular permeation. Conjugation of lipophilic groups like cholesterol (Manoharan, 2002) and lauric and lithocholic acid derivatives with C32 functionality (Lorenz *et al.*, 2004), have been shown to improve cellular uptake. Moreover binding of steroid conjugated oligonucleotides to different lipoproteins in the bloodstream, such as LDL, protect their integrity and govern their biodistribution (Rump *et al.,* 2000). Cholesterol attached to anti-sense molecules (Bijsterbosch *et al.*, 2001) and aptamers (Rusconi *et al.*, 2004) has also been shown to stabilize oligonucleotides by allowing binding to lipoproteins. Cholesterol has been demonstrated to enhance uptake and serum stability of siRNAs *in vitro* (Lorenz *et al.*, 2004) and *in vivo* (Soutschek *et al.*, 2004). Additionally, a number of small molecules like SB-435495 (Blackie *et al.,* (2002), Isradipine (Oravcova *et al.*, 1994), amlodipine (Oravcova *et al.*, 1994) and 2,2',4,4',5,5'-hexachlorobiphenyl (Borlakoglu *et al.*, 1990) could enhance cellular uptake, and improve nuclease resistance by promoting lipoprotein association.

The present methods and kits may be employed for high volume screening. A library of synthetic miRNAs and/or miRNA inhibitors can be created using methods of the invention. This library may then be used in high throughput assays, including microarrays. Specifically contemplated by the present inventors are chip-based nucleic acid technologies such as those described by Ziauddin and Sabatini (2001). Briefly, nucleic acids can be immobilized on solid supports. Cells can then be overlaid on the solid support and take up the nucleic acids at the defined locations. The impact on the cells can then be measured to identify cocktails that are having a desirable effect.

C. Labeling and Labeling Techniques

In some embodiments, the present invention concerns miRNA that are labeled, such as for screening assays to evaluate the therapeutic or diagnostic relevance of a particular miRNA species. It is contemplated that miRNA may first be isolated (either from a cell in which the miRNA is endogenous to the cell or from a cell in which miRNA is exogenous to the cell) and/or purified prior to labeling. This may achieve a reaction that more efficiently labels the miRNA, as opposed to other RNA in a sample in which the miRNA is not isolated or purified prior to labeling. In many embodiments of the invention, the label is non-radioactive. Generally, nucleic acids may be labeled by adding labeled nucleotides (one-step process) or adding nucleotides and labeling the added nucleotides (two-step process).

Moreover, miRNA may be labeled. Such nucleotides include those that can be labeled with a dye, including a fluorescent dye, or with a molecule such as biotin. Labeled nucleotides are readily available; they can be acquired commercially or they can be synthesized by reactions known to those of skill in the art.

1. Nucleotides for Labeling

Nucleotides for labelling are not naturally occurring nucleotides, but instead, refer to prepared nucleotides that have a reactive moiety on them. Specific reactive functionalities of interest include: amino, sulfhydryl, sulfoxyl, aminosulfhydryl, azido, epoxide, isothiocyanate, isocyanate, anhydride, monochlorotriazine, dichlorotriazine, mono-or dihalogen substituted pyridine, mono- or disubstituted diazine, maleimide, epoxide, aziridine, sulfonyl halide, acid halide, alkyl halide, aryl halide, alkylsulfonate, N-hydroxysuccinimide ester, imido ester, hydrazine, azidonitrophenyl, azide, 3-(2-pyridyl dithio)-propionamide, glyoxal, aldehyde, iodoacetyl, cyanomethyl ester, p-nitrophenyl ester, o-nitrophenyl ester, hydroxypyridine ester, carbonyl imidazole, and the other such chemical groups. In some embodiments, the reactive functionality may be bonded directly to a nucleotide, or it may be bonded to the nucleotide through a linking group. The functional moiety and any linker cannot substantially impair the ability of the nucleotide to be added to the miRNA or to be labeled. Representative linking groups include carbon containing linking groups, typically ranging from about 2 to 18, usually from about 2 to 8 carbon atoms, where the carbon containing linking groups may or may not include one or more heteroatoms, e.g. S, O, N etc., and may or may not include one or more sites of unsaturation. Of particular interest in many embodiments are alkyl linking groups, typically lower alkyl linking groups of 1 to 16, usually 1 to 4 carbon atoms, where the linking groups may include one or more sites of unsaturation. The functionalized nucleotides (or primers) used in the above methods of functionalized target generation may be fabricated using known protocols or purchased from commercial vendors, e.g., Sigma, Roche, Ambion, and NEN. Functional groups may be prepared according to ways known to those of skill in the art, including the representative information found in U.S. Pat. Nos. 4,404,289; 4,405,711; 4,337,063 and 5,268,486, and Br. Pat. No. 1,529,202.

Amine-modified nucleotides are used in several embodiments of the invention. The amine-modified nucleotide is a nucleotide that has a reactive amine group for attachment of the label. It is contemplated that any ribonucleotide (G, A, U, or C) or deoxyribonucleotide (G,A,T, or C) can be modified for labeling. Examples include, but are not limited to, the following modified ribo- and deoxyribo-nucleotides: 5-(3-aminoallyl)-UTP; 8-[(4-amino)butyl]-amino-ATP and 8-[(6-amino)butyl]-amino-ATP; N^6-(4-amino)butyl-ATP, N^6-(6-amino)butyl-ATP, N^4-[2,2-oxy-*bis*-(ethylamine)]-CTP; N^6-(6-Amino)hexyl-ATP; 8-[(6-Amino)hexyl]-amino-ATP; 5-propargylamino-CTP, 5-propargylamino-UTP; 5-(3-aminoallyl)-dUTP; 8-[(4-amino)butyl]-amino-dATP and 8-[(6-amino)butyl]-amino-dATP; N^6-(4-amino)butyl-dATP, N^6-(6-amino)butyl-dATP, N^4-[2,2-oxy-*bis*-(ethylamine)]-dCTP; N^6-(6-Amino)hexyl-dATP; 8-[(6-Amino)hexyl]-amino-dATP; 5-propargylamino-dCTP, and 5-propargylamino-dUTP. Such nucleotides can be prepared according to methods known to those of skill in the art. Moreover, a person of ordinary skill in the art could prepare other nucleotide entities with the same amine-modification, such as a 5-(3-aminoallyl)-CTP, GTP, ATP, dCTP, dGTP, dTTP, or dUTP in place of a 5-(3-aminoallyl)-UTP.

2. Labeling Techniques

In some embodiments, nucleic acids are labeled by catalytically adding to the nucleic acid an already labeled nucleotide or nucleotides. One or more labeled nucleotides can be added to miRNA molecules. See U.S Patent 6,723,509.

In other embodiments, an unlabeled nucleotide or nucleotides is catalytically added to an miRNA, and the unlabeled nucleotide is modified with a chemical moiety that enables it to be subsequently labeled. In embodiments of the invention, the chemical moiety is a reactive amine such that the nucleotide is an amine-modified nucleotide.

Examples of amine-modified nucleotides are well known to those of skill in the art, many being commercially available such as from Ambion, Sigma, Jena Bioscience, and TriLink.

In contrast to labeling of cDNA during its synthesis, the issue for labeling miRNA is how to label the already existing molecule. The present invention concerns the use of an enzyme capable of using a di- or tri-phosphate ribonucleotide or deoxyribonucleotide as a substrate for its addition to an miRNA, a small RNA molecule. Moreover, in specific embodiments, it involves using a modified di- or tri-phosphate ribonucleotide, which is added to the 3' end of an miRNA. The source of the enzyme is not limiting. Examples of sources for the enzymes include yeast, gram-negative bacteria such as E. coli, lactococcus lactis, and sheep pox virus.

Enzymes capable of adding such nucleotides include, but are not limited to, poly(A) polymerase, terminal transferase, and polynucleotide phosphorylase. In specific embodiments of the invention, ligase is contemplated as NOT being the enzyme used to add the label, and instead, a non-ligase enzyme is employed.

Poly(A) polymerase has been cloned from a number of organisms from plants to humans. It has been shown to catalyze the addition of homopolymer tracts to RNA (Martin *et al.*, *RNA*, 4(2):226-30, 1998).

Terminal transferase catalyzes the addition of nucleotides to the 3' terminus of a nucleic acid.

Polynucleotide phosphorylase can polymerize nucleotide diphosphates without the need for a primer.

3. Labels

Labels on miRNA or miRNA probes may be colorimetric (includes visible and UV spectrum, including fluorescent), luminescent, enzymatic, or positron emitting (including radioactive). The label may be detected directly or indirectly. Radioactive labels include ^{125}I, ^{32}P, ^{33}P, and ^{35}S. Examples of enzymatic labels include alkaline phosphatase, luciferase, horseradish peroxidase, and β-galactosidase. Labels can also be proteins with luminescent properties, e.g., green fluorescent protein and phicoerythrin.

The colorimetric and fluorescent labels contemplated for use as conjugates include, but are not limited to, Alexa Fluor dyes, BODIPY dyes, such as BODIPY FL; Cascade Blue; Cascade Yellow; coumarin and its derivatives, such as 7-amino-4-

methylcoumarin, aminocoumarin and hydroxycoumarin; cyanine dyes, such as Cy3 and Cy5; eosins and erythrosins; fluorescein and its derivatives, such as fluorescein isothiocyanate; macrocyclic chelates of lanthanide ions, such as Quantum Dye™; Marina Blue; Oregon Green; rhodamine dyes, such as rhodamine red, tetramethylrhodamine and rhodamine 6G; Texas Red; , fluorescent energy transfer dyes, such as thiazole orange-ethidium heterodimer; and, TOTAB.

Specific examples of dyes include, but are not limited to, those identified above and the following: Alexa Fluor 350, Alexa Fluor 405, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 500. Alexa Fluor 514, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 610, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, and, Alexa Fluor 750; amine-reactive BODIPY dyes, such as BODIPY 493/503, BODIPY 530/550, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY 630/650, BODIPY 650/655, BODIPY FL, BODIPY R6G, BODIPY TMR, and, BODIPY-TR; Cy3, Cy5, 6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, Renographin, ROX, SYPRO, TAMRA, 2',4',5',7'-Tetrabromosulfonefluorescein, and TET.

Specific examples of fluorescently labeled ribonucleotides are available from Molecular Probes, and these include, Alexa Fluor 488-5-UTP, Fluorescein-12-UTP, BODIPY FL-14-UTP, BODIPY TMR-14-UTP, Tetramethylrhodamine-6-UTP, Alexa Fluor 546-14-UTP, Texas Red-5-UTP, and BODIPY TR-14-UTP. Other fluorescent ribonucleotides are available from Amersham Biosciences, such as Cy3-UTP and Cy5-UTP.

Examples of fluorescently labeled deoxyribonucleotides include Dinitrophenyl (DNP)-11-dUTP, Cascade Blue-7-dUTP, Alexa Fluor 488-5-dUTP, Fluorescein-12-dUTP, Oregon Green 488-5-dUTP, BODIPY FL-14-dUTP, Rhodamine Green-5-dUTP, Alexa Fluor 532-5-dUTP, BODIPY TMR-14-dUTP, Tetramethylrhodamine-6-dUTP, Alexa Fluor 546-14-dUTP, Alexa Fluor 568-5-dUTP, Texas Red-12-dUTP, Texas Red-5-dUTP, BODIPY TR-14-dUTP, Alexa Fluor 594-5-dUTP, BODIPY 630/650-14-dUTP, BODIPY 650/665-14-dUTP; Alexa Fluor 488-7-OBEA-dCTP, Alexa Fluor 546-16-OBEA-dCTP, Alexa Fluor 594-7-OBEA-dCTP, Alexa Fluor 647-12-OBEA-dCTP.

It is contemplated that nucleic acids may be labeled with two different labels. Furthermore, fluorescence resonance energy transfer (FRET) may be employed in methods of the invention (*e.g.*, Klostermeier *et al.*, 2002; Emptage, 2001; Didenko, 2001).

Alternatively, the label may not be detectable per se, but indirectly detectable or allowing for the isolation or separation of the targeted nucleic acid. For example, the label could be biotin, digoxigenin, polyvalent cations, chelator groups and the other ligands, include ligands for an antibody.

4. Visualization Techniques

A number of techniques for visualizing or detecting labeled nucleic acids are readily available. The reference by Stanley T. Crooke, 2000 has a discussion of such techniques (Chapter 6). Such techniques include, microscopy, arrays, Fluorometry, Light cyclers or other real time PCR machines, FACS™ analysis, scintillation counters, Phosphoimagers™, Geiger™ counters, MRI, CAT, antibody-based detection methods (Westerns, immunofluorescence, immunohistochemistry), histochemical techniques, HPLC (Griffey *et al.*, 1997, spectroscopy, capillary gel electrophoresis (Cummins *et al.*, 1996), spectroscopy; mass spectroscopy; radiological techniques; and mass balance techniques.

When two or more differentially colored labels are employed, fluorescent resonance energy transfer (FRET) techniques may be employed to characterize the dsRNA. Furthermore, a person of ordinary skill in the art is well aware of ways of visualizing, identifying, and characterizing labeled nucleic acids, and accordingly, such protocols may be used as part of the invention. Examples of tools that may be used also include fluorescent microscopy, a BioAnalyzer™, a plate reader, Storm™ (Molecular Dynamics), Array Scanner™, FACS™ (fluorescent activated cell sorter), or any instrument that has the ability to excite and detect a fluorescent molecule.

C. Array Preparation

The present invention can be employed with miRNA arrays, which are ordered macroarrays or microarrays of nucleic acid molecules (probes) that are fully or nearly complementary or identical to a plurality of miRNA molecules or precursor miRNA molecules and that are positioned on a support material in a spatially separated organization. Macroarrays are typically sheets of nitrocellulose or nylon upon which probes have been spotted. Microarrays position the nucleic acid probes more densely such that up to 10,000 nucleic acid molecules can be fit into a region typically 1 to 4 square centimeters. Microarrays can be fabricated by spotting nucleic acid molecules, *e.g.*, genes, oligonucleotides, etc., onto substrates or fabricating oligonucleotide sequences in situ on a substrate. Spotted or fabricated nucleic acid molecules can be applied in a high density matrix pattern of up to about 30 non-identical nucleic acid molecules per square centimeter or higher, e.g. up to about 100 or even 1000 per square centimeter. Microarrays typically use coated glass as the solid support, in contrast to the nitrocellulose-based material of filter arrays. By having an ordered array of miRNA-complementing nucleic acid samples, the position of each sample can be tracked and linked to the original sample. A variety of different array devices in which a plurality of distinct nucleic acid probes are stably associated with the surface of a solid support are known to those of skill in the art. Useful substrates for arrays include nylon, glass and silicon Such arrays may vary in a number of different ways, including average probe length, sequence or types of probes, nature of bond between the probe and the array surface, e.g. covalent or non-covalent, and the like.

Representative methods and apparatus for preparing a microarray have been described, for example, in U.S. Patent Nos. 5,143,854; 5,202,231; 5,242,974; 5,288,644; 5,324,633; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,432,049; 5,436,327; 5,445,934; 5,468,613; 5,470,710; 5,472,672; 5,492,806; 5,525,464; 5,503,980; 5,510,270; 5,525,464; 5,527,681; 5,529,756; 5,532,128; 5,545,531; 5,547,839; 5,554,501; 5,556,752; 5,561,071; 5,571,639; 5,580,726; 5,580,732; 5,593,839; 5,599,695; 5,599,672; 5,610;287; 5,624,711; 5,631,134; 5,639,603; 5,654,413; 5,658,734; 5,661,028; 5,665,547; 5,667,972; 5,695,940; 5,700,637; 5,744,305; 5,800,992; 5,807,522; 5,830,645; 5,837,196; 5,871,928; 5,847,219; 5,876,932; 5,919,626; 6,004,755; 6,087,102; 6,368,799; 6,383,749; 6,617,112; 6,638,717; 6,720,138, as well as WO 93/17126; WO 95/11995; WO 95/21265; WO 95/21944; WO 95/35505; WO 96/31622; WO 97/10365; WO 97/27317; WO 99/35505; WO 09923256; WO 09936760; WO0138580; WO 0168255; WO 03020898; WO 03040410; WO 03053586; WO 03087297; WO 03091426; WO03100012; WO 04020085; WO 04027093; EP 373 203; EP 785 280; EP 799 897 and UK 8 803 000.

It is contemplated that the arrays can be high density arrays, such that they contain 100 or more different probes. It is contemplated that they may contain 1000, 16,000, 65,000, 250,000 or 1,000,000 or more different probes. The probes can be directed to targets in one or more different organisms. The oligonucleotide probes range from 5 to 50, 5 to 45, 10 to 40, or 15 to 40 nucleotides in length in some embodiments. In certain embodiments, the oligonucleotide probes are 20 to 25 nucleotides in length.

The location and sequence of each different probe sequence in the array are generally known. Moreover, the large number of different probes can occupy a relatively small area providing a high density array having a probe density of generally greater than about 60, 100, 600, 1000, 5,000, 10,000, 40,000, 100,000, or 400,000 different oligonucleotide probes per cm^2. The surface area of the array can be about or less than about 1, 1.6, 2, 3, 4, 5, 6, 7, 8, 9, or 10 cm^2.

Moreover, a person of ordinary skill in the art could readily analyze data generated using an array. Such protocols are disclosed above, and include information found in WO 9743450; WO 03023058; WO 03022421; WO 03029485; WO 03067217; WO 03066906; WO 03076928; WO 03093810; WO 03100448A1.

D. Sample Preparation

It is contemplated that the miRNA of a wide variety of samples can be analyzed using assays described herein. While endogenous miRNA is contemplated for use with some embodiments, recombinant miRNA—including nucleic acids that are complementary or identical to endogenous miRNA or precursor miRNA—can also be handled and analyzed as described herein. Samples may be biological samples, in which case, they can be from blood, tissue, organs, semen, saliva, tears, other bodily fluid, hair follicles, skin, or any sample containing or constituting biological cells. Alternatively, the sample may not be a biological sample, but be a chemical mixture, such as a cell-free reaction mixture (which may contain one or more biological enzymes).

E. Hybridization

After the array is prepared and the miRNA in the sample is labeled, the population of target nucleic acids is contacted with the array under hybridization conditions, where such conditions can be adjusted, as desired, to provide for an optimum level of specificity in view of the particular assay being performed. Suitable hybridization conditions are well known to those of skill in the art and reviewed in Sambrook *et al.*, 1989 and WO 95/21944. Of particular interest in many embodiments is the use of stringent conditions during hybridization. Stringent conditions are known to those of skill in the art.

It is specifically contemplated that a single array may be contacted with multiple samples. The samples may be labeled with different labels to distinguish the samples. For example, a single array can be contacted with a tumor tissue sample labeled with Cy3, and normal tissue sample labeled with Cy5. Differences between the samples for particular miRNAs corresponding to probes on the array can be readily ascertained and quantified.

The small surface area of the array permits uniform hybridization conditions, such as temperature regulation and salt content. Moreover, because of the small area occupied by the high density arrays, hybridization may be carried out in extremely small fluid volumes (e.g., about 250 μl or less, including volumes of about or less than about 5, 10, 25, 50, 60, 70, 80 ,90, 100 μl, or any range derivable therein). In small volumes, hybridization may proceed very rapidly.

F. Differential Expression Analyses

Arrays can be used to detect differences between two samples. This can also be used for diagnostic purposes. Specifically contemplated applications include identifying and/or quantifying differences between miRNA from a sample that is normal and from a sample that is not normal or between two differently treated samples. Also, miRNA may be compared between a sample believed to be susceptible to a particular disease or condition and one believed to be not susceptible or resistant to that disease or condition. A sample that is not normal is one exhibiting phenotypic trait(s) of a disease or condition or one believed to be not normal with respect to that disease or condition. It may be compared to a cell that is normal with respect to that disease or condition. Phenotypic traits include symptoms of, or susceptibility to, a disease or condition of which a component is or may or may not be genetic.

G. Cell Assays To Identify miRNAs with Ties to Disease

Specifically contemplated applications include identifying miRNAs that contribute to cellular processes that are themselves parts of a disease or might otherwise be associated with a particular disease state. Also, miRNA functions may be compared between a sample believed to be susceptible to a particular disease or condition and one believed to be not susceptible or resistant to that disease or condition. It is specifically contemplated that RNA molecules of the present invention can be used to treat any of the diseases or conditions discussed in the previous section or modulate any of the cellular pathways discussed in the previous section.

Specifically contemplated applications include identifying miRNAs that contribute to cellular processes that are themselves parts of a disease or might otherwise be associated with a particular disease state. Also, miRNA functions may be compared between a sample believed to be susceptible to a particular disease or condition and one believed to be not susceptible or resistant to that disease or condition.

AIDS, autoimmune diseases (rheumatoid arthritis, multiple sclerosis, diabetes—insulin-dependent and non-independent, systemic lupus erythematosus and Graves disease); cancer (*e.g.,* malignant, benign, metastatic, precancer); cardiovascular diseases (heart disease or coronary artery disease, stroke—ischemic and hemorrhagic, and rheumatic heart disease); diseases of the nervous system; and infection by pathogenic microorganisms (Athlete's Foot, Chickenpox, Common cold, Diarrheal diseases, Flu, Genital herpes, Malaria, Meningitis, Pneumonia, Sinusitis, Skin diseases, Strep throat, Tuberculosis, Urinary tract infections, Vaginal infections, Viral hepatitis); inflammation (allergy, asthma); prion diseases (*e.g.,* CJD, kuru, GSS, FFI).

Moreover, miRNA can be evaluated with respect to the following diseases, conditions, and disorders: Abdominal Adhesions; Anal Abscess; Brain Abscess; Peritonsillar Abscess; Absence Seizures; Achalasia; Acne; Acoustic Neuroma; Acquired Immunodeficiency Syndrome (AIDS); Acrochordon; Actinic Keratosis; Adenocarcinoma of the Lung; ADHD; Adult-Onset Diabetes; Aero-Otitis; Age Spots; Age-Related Hearing Loss; Age-Related Macular Degeneration; Age-Related Vision Change (Presbyopia); Agoraphobia; Alcohol Withdrawal; Alcoholism; Allergen Immunotherapy; Allergic Rhinitis; Allergies; Alopecia (Areata, Hereditary-Patterned, and Traumatic); Altitude Sickness; Alzheimer's Disease; Amaurotic Familial Infantile Idiocy; Amblyopia; Amenorrhea; Amyloidosis; Amyotrophic Lateral Sclerosis (ALS); Anaphylaxis; Androgenetic Alopecia; Anemia (Aplastic, Hemolytic, Pernicious, and Sickle Cell); Angina; Angiomas, Spider; Angioplasty; Ankylosing Spondylitis; Anorexia Nervosa; Anovulatory Bleeding; Antibiotic-Associated Diarrhea; Antiphospholipid Antibody Syndrome; Antisocial Personality Disorder; Anus Fissure, Fistula, Hemorrhoids, Anus Itch, Stricture; Anxiety Disorders (Generalized, Obsessive-Compulsive Disorder, Panic Disorder, Phobia, and Post-Traumatic Stress Disorder); Aortic Aneurysm; Aortic Arch Syndrome; Appendicitis; Arrhythmias, Cardiac; Arteritis, Takayasu's; Arthritic Diseases (Ankylosing Spondylitis, Gout, Infectious, Juvenile, Osteoarthritis, Pseudogout, Psoriatic Arthritis, and Rheumatoid); Asbestosis; Ascending Cholangitis; Asteatotic Eczema; Asthma; Astigmatism; Asymptomatic Bacteriuria; Ataxia, Friedreich's; Atherosclerosis; Athlete's Foot; Atopic Dermatitis; Atrial Fibrillation; Atrophic Vaginitis; Attention-Deficit Hyperactivity Disorder; Autism; Autoimmune Diseases (Celiac Disease, Crohn's Disease, Diabetes Mellitus, Type 1 (Insulin-Dependent; Juvenile-Onset) , Diabetes Mellitus, Type 2 (Non-Insulin-Dependent; Adult-Onset), Graves' Disease, Hyperthyroidism, Immune Thrombocytopenic Purpura, Lupus, Myasthenia Gravis, Polyarteritis Nodosa, Rheumatoid Arthritis, Scleroderma, Takayasu's Arteritis, and Ulcerative Colitis); B12 Deficiency; Bacillary Dysentery; Bacterial Gastroenteritis; Bacterial Vaginosis; Balanitis; Baldness, Hereditary- Patterned; Barber's Itch; Barotitis; Barotrauma; Bartholin's Gland Cyst; Basal-Cell Carcinoma; Bed-Wetting; Bedsores; Behcet's Syndrome; Bell's Palsy; Bends; Benign Prostatic Hyperplasia; Bile-Duct Diseases; Biliary Colic; Biopsy; Bipolar Disorder; Bladder conditions (Infection; Interstitial Cystitis; Prolapse; Urethritis; Urinary Incontinence; Urinary Tract Infection); Blepharitis; Blepharoptosis; Blighted Ovum; Friction Blisters; Blood Pressure, High; Boils; Bone diseases and conditions (Osteoporosis;Paget's Disease); Bone Yaws; Borderline Personality Disorder; Bornholm Disease; Botulism; Bowel Obstruction; Bradycardia; Bronchitis; Bulimia Nervosa; Bunion; Bursitis; C. Difficile Colitis; Calcaneal Apophysitis; Calcium Pyrophosphate Deposition Disease; Campylobacteriosis; Cancer; Candidiasis; Carbon-Monoxide Poisoning; Carbuncles; Cardiac Arrhythmias (Atrial Fibrillation, Bradycardia); Cardiomyopathy; Carpal Tunnel Syndrome; Cataracts; Cellulitis; Central Serous Retinopathy; Cerebral Palsy; Cerebromacular Degeneration; Cerumen Impaction; Cervicitis, Nabothian Cysts, Cervical Polyps, Cervical Warts; Chalazion; Chickenpox; Chlamydia; Chloasma; Cholangitis; Cholecystitis; Cholesteatoma; Chondromalacia; Chorea; Choroidal Melanoma; Chronic Bronchitis; Chronic Fatigue Syndrome; Chronic Hepatitis; Chronic Leukemia; Chronic Obstructive Pulmonary Disease; Chronic Otitis Media; Cirrhosis; Cluster Headache; Cogan's Syndrome; Cold, Common; Colic, Biliary; Pseudomembranous Colitis, Ulcerative Colitis, Collapsed Lung; Collarbone Fracture; Coma; Complex Regional Pain Syndrome; Congestive Heart Failure; Conjunctivitis; Constipation; Contact Dermatitis; Conversion Disorder; COPD; Cornea Abrasion, Cornea Keratitis; Corns; Coronary Artery Disease; Creutzfeldt-Jakob Disease; Crossed Eyes; Croup; Cryptorchidism; Cystic Fibrosis; Interstitial Cystitis; Cystocele; Cysts; Cytomegalovirus infection; Dacryocystitis; Dandruff; Decompression Sickness; Decubitus Ulcers; Delirium Tremens; Delusional Disorder; Dementia; Depressive Disorders (Bipolar Disorder, Dysthymia, Major Depression, Manic Depression, Postpartum Depression); Dermatitis; Dermatofibroma; Dermatomyositis; Detached Retina; Developmental Dysplasia of the Hip; Deviated Septum; Devil's Grip; Diabetes (Gestational Diabetes; Type 1 Diabetes (Insulin-Dependent; Juvenile); Type 2 Diabetes (Non-Insulin-Dependent; Adult-Onset); Hypoglycemia, Ketoacidosis, Nephropathy, Neuropathies, Retinopathy) Antibiotic-Associated Diarrhea; Diplopia; Herniated Disk; Dislocated Lens; Hip Dislocation (Developmental); Diverticulitis; Diverticulosis; Dizziness; Doerderland's Vaginitis; Double Vision; Down Syndrome; Drooping Eyelid; Dry Skin; Sun-Damaged Skin; Dry-Eye Syndrome; Duck-Foot; Dysautonomia, Familial; Dysfunctional Uterine Bleeding; Dyslexia; Dyspareunia; Dysthymia; Dysuria; Eating Disorders (Anorexia Nervosa, Bulimia Nervosa); Eclampsia; Eczema; Edema; Emphysema; Encephalitis; Encopresis; End-Stage Renal Disease; Endocarditis; Endometriosis; Endophthalmitis; Endoscopy; Enlarged Prostate; Enuresis; Epidemic Benign Dry Pleurisy; Epididymitis; Epiglottitis; Epilepsy; Epistaxis; Erectile Dysfunction; Erythema Infectiosum; Esophagitis; Esophagus Achalasia; Esophagitis; Essential Hypertension; Essential Tremor; Ewing's Sarcoma; Familial Dysautonomia; Farsightedness; Febrile Seizures; Fecal Incontinence; Fever; Fever-Induced Seizures; Fibroids; Fibromyalgia; Fifth Disease; Filiform Warts; Flat Warts; Flatulence; Flu; Focal Seizures; Food Allergy; Food Poisoning; Forefoot Neuroma; Fragile X Syndrome; Friction Blisters; Friedreich's Ataxia; Frostbite; Fungal Infections (Athlete's Foot, Brain Abscess, Infectious Arthritis, Jock Itch, Onychomycosis, Ringworm, Swimmer's Ear, Tinea

Cruris, Tinea Unguium, Tinea Versicolor); Furuncle; Gallstones; Gardnerella Vaginitis; Gastritis; Gastrocnemius Strain; Gastroenteritis; Gastroesophageal Reflux Disease; Gastrointestinal Amebiasis; Generalized Anxiety Disorder; Generalized Barotrauma; Genital Herpes; Genital Warts; GERD; Germ Cell Tumors, Extragonadal; Giant Cell Arteritis; Giardiasis; Glaucoma; Glomerulonephritis; Gluten-Sensitive Enteropathy; GM2 Gangliosidosis; Gonorrhea; Gout; Grand Mal Seizures; Graves' Disease; Graves' Ophthalmopathy; Guillain-Barré Syndrome; Hammertoe; Hay Fever; Headache; Hearing Loss; Heart Attack;; Heat Stroke; Heel Spur; Heloma; Spider Hemangiomas; Hematoma; Hematuria; Hemochromatosis; Hemolytic Anemia; Hemophilia; Hemorrhagic Stroke; Subarachnoid Hemorrhagic Stroke; Hemorrhoids; Hepatitis A; Hepatitis B; Hepatitis C; Hereditary-Patterned Baldness; Hernia; Herniated Disk; High Blood Pressure; High Cholesterol; Hirsutism; Histiocytosis X; HIV/AIDS; Hordeolum; Human Papilloma Virus (HPV); Huntington's Disease; Hydatidiform Mole; Hydrocephalus; Hyperactivity; Hypercholesterolemia; Hyperkeratosis; Hyperopia; Hypertension; Ocular Hypertension; Secondary Hypertension; Hypertensive Retinopathy; Hyperthermia; Hyperthyroidism; Hypochondriasis; Hypoglycemia; Hypoparathyroidism; Hypothyroidism; IBS; ICD; Ichthyosis; Immune Thrombocytopenic Purpura; Impetigo; Impotence; Incontinence; Infantile Ganglioside Lipidosis; Infectious Arthritis; Infectious Mononucleosis; Infertility; Inflammatory Bowel Disease; Inguinal Hernia; Insomnia; Intercerebral Hemorrhage; Interdigital Neuroma; Intermetatarsal Neuroma; Intermittent Claudication; Interstitial Cystitis; Intestinal Obstruction; Iron Deficiency; Irritable Bowel Syndrome; Juvenile Arthritis; Kaposi's Sarcoma; Kawasaki Syndrome; Keloids; Keratitis; Actinic Keratosis; Labyrinthitis; Lactose Intolerance; Lacunar Stroke; Langerhans' Cell Histiocytosis; Laryngitis; Laryngotracheitis; Lateral Epicondylitis; Latex Allergy; Lazy Eye; Lead Poisoning; Intermittent Claudication; Restless Legs Syndrome; Shin Splints; Leg Strain; Cataract; Dislocated Lens; Leukemia; Lice; Lichen Simplex Chronicus; Cirrhosis; Hepatitis; Liver Spots; Lockjaw; Lou Gehrig's Disease; Lupus Erythematosus, Systemic; Lyme Disease; Lymphedema; Lymphoma; Macular Degeneration; Malabsorption Syndromes; Malaria; Male Pattern Baldness; Malignant Hyperthermia; Manic Depression; Marfan's Syndrome; Mastoiditis; Measles; Meckel's Diverticulum; Melasma; Meniere's Disease; Meningitis; Menopause; Mental Retardation; Phenylketonuria; Migraine; Miscarriage; Mitral-Valve Prolapse; Mittelschmerz;

Molar Pregnancy; Molluscum Contagiosum; Mononucleosis; Morton's Neuroma; Mosaic Warts; Motor Tics; Mucocutaneous Lymph Node Syndrome; Multiple Sclerosis; Mumps; Muscular Dystrophy; Musculoskeletal Disorders (Fibromyalgia, Giant Cell Arteritis, Gout, Infectious Arthritis, Muscular Dystrophy, Myositis, Osteoarthritis, Osteoporosis, Paget's Disease Of Bone, Polymyalgia Rheumatica, Pseudogout, Reflex Sympathetic Dystrophy, Rheumatoid Arthritis, Scleroderma, Systemic Lupus Erythematosus, Tendonitis); Myasthenia Gravis; Myocardial Infarction; Myocarditis; Myopia; Myositis; Nail Felon; Onycholysis; Onychomycosis; Paronychia; Subungual Hematoma; Narcolepsy; Nasal Polyps; Nausea; Nearsightedness; Needle Biopsy; Nephrectomy; Nephroblastoma; Nephrolithiasis; Nephropathy, Diabetic; Neuritis, Retrobulbar; Neuroblastoma; Neuromuscular Disorders; Neuropathies; Guillain-Barre Syndrome; Retrobulbar; Nevi; Nevus Flammeus; Nevus Simplex; Nocturnal Enuresis; Non-Tropical Sprue; Obesity; Obsessive-Compulsive Disorder; Occupational Hearing Loss; Ocular Hypertension; Ocular Rosacea; Onycholysis; Onychomycosis; Glaucoma; Retrobulbar Neuritis; Optic Nerve Swelling; Orbit Fracture; Orchitis; Osgood-Schlatter Disease; Osteoarthritis; Osteoporosis; Osteosarcoma; Otitis Externa; Otitis Media; Chronic Otitis Media; Otosclerosis; Ototoxicity; Pelvic Inflammatory Disease; Polycystic Ovary Syndrome; Painful-Bladder Syndrome; Pancreatitis; Panic Disorder; Papilledema; Paraphimosis; Parkinson's Disease; Paronychia; Partial Seizures; PCL Injuries; Pedunculated Warts; Pelvic Relaxation; Paraphimosis; Peyronie's Disease; Peptic Ulcer; Perforated Eardrum; Pericarditis; Perimenopause; Peripheral Vascular Disease; Peritonsillar Abscess; Persistent Vegetative State; Personality Disorders; Petit Mal Seizures; Peyronie's Disease; Pharyngitis; Pharynx Cancer; Phenylketonuria; Phimosis; Phobia; Photosensitivity; Pigmentation Disorders (Chloasma, Melasma, Vitiligo); Piles; Pinkeye; Pityriasis Rosea; PKU; Plague; Plantar Fasciitis; Plantar Warts; Plantaris Strain; Pleurisy; Pleurodynia; PMS; Pneumoconiosis; Pneumonectomy; Pneumonia; Pneumothorax; Lead Poisoning; Polio; Poliomyelitis; Polyarteritis Nodosa; Polychondritis; Polymyalgia Rheumatica; Polymyositis; Colonic Polyps; Nasal Polyps; Vocal Cord Polyps; Port-Wine Stain; Post-Polio Syndrome; Postinfectious Thrombocytopenia; Postpartum Depression; Preeclampsia; Pregnancy-Induced Hypertension; Premenstrual Syndrome; Pressure Sores; Primary Sclerosing Cholangitis; Prolapse; Enlarged Prostate; Acute Prostatitis; Chronic Prostatitis; Pruritus Ani; Pseudogout; Psoriasis; Psoriatic Arthritis; Ptosis;

Pulseless Disease; Pyelonephritis; Quadriceps Strain; Quinsy; Rash; Raynaud's Phenomenon; Rectal Itch; Rectocele; Reflex Sympathetic Dystrophy; Renal Failure; Respiratory Disorders Respiratory Syncytial Virus; Retina Detachment; Retinitis Pigmentosa; Retinopathy; Retrobulbar Neuritis; Reye's Syndrome; Rhabdomyosarcoma; Rheumatoid Arthritis; Allergic Rhinitis; Viral Rhinitis (Common Cold); Riley-Day Syndrome; Ringworm; Rocky Mountain Spotted Fever; Rosacea; Rubeola; Mumps; Salivary Gland Disorders; Salmon Patch; Sarcoidosis; Scabies; Scarlet Fever; Scars; Schizophrenia; Schizotypal Personality Disorder; Sciatica; Scleritis; Scleroderma; Scoliosis; Sebaceous Cysts; Seborrhea; Seborrheic Keratoses; Secondary Hypertension; Seizures; Sexual Dysfunction; Sexually Transmitted Diseases; Shigellosis; Shingles; Sialadenitis; Sialadenosis; Sialolithiasis; Sickle-Cell Anemia; Siderosis; Silicosis; Sinus Cancer; Sjögren's Syndrome; Sleep Disorders; Smallpox; Social Anxiety Disorder; Solar Lentigo; Somatoform Disorders (Hypochondriasis, Somatization Disorder); Somnambulism; Spastic Colon; Spider Veins; Spina Bifida; Spinal Cord Trauma; Spontaneous Abortion; Stasis Dermatitis; Strabismus; Strep Throat; Streptococcal Toxic Shock Syndrome; Stroke; Subarachnoid Hemorrhage; Transient Ischemic Attack; Stuttering; Subungual Hematoma; Sun Allergy; Sun-Damaged Skin; Sylvest's Disease; Systemic Lupus Erythematosus; Systemic Sclerosis; Tachycardia; Takayasu's Arteritis; Tay-Sachs Disease; Tear-Duct Infection; Telogen Effluvium; Temporal Arteritis; Tendonitis; Tennis Elbow; Tension Headache; Testicular Torsion; Undescended Testicles; Tetanus; Thrombocytopenia; Thrombophlebitis; Thrombotic Stroke; Tinea; Tinnitus; Tonsillitis; Torsional Deformities; Toxemia Of Pregnancy; Toxic Shock Syndrome, Streptococcal; Toxoplasmosis; Trichomoniasis; Trigeminal Neuralgia (Tic Douloureux); Tuberculosis; Tylosis; Ulcer; Urethritis; Urinary Tract disorders and conditions; Uroliniasis; Urticaria; Uterine disorders; Uterine Prolapse; Uveitis; Vaginitis; Bacterial (Gardnerella) Vaginosis; Varicella; Varices, Esophageal; Varicose Veins;; Vascular Disorders (Hypertension, Intermittent Claudication, Peripheral Vascular Disease, Polyarteritis Nodosa, Raynaud's Phenomenon, Takayasu's Arteritis, Thrombophlebitis, Vasculitis, Wegener's Granulomatosis); Vein Inflammation; Varicose Veins; Vertigo; Vestibular Schwannoma; Viral Rhinitis; Vitamin B12 Deficiency; Vitiligo; Vocal Tics; Vocal-Cord Disorders; Common Warts; Genital Warts; Plantar Warts; Water On The Brain; Wax Blockage Of Ear Canal; Esophageal Webs; Werlhof's Disease; Wrinkles; Yersinia Pestis Infection. It is contemplated that such diseases can be diagnosed or treated using a nucleic acids of the invention that correspond to miRNAs.

Cancers that may be evaluated, diagnosed, and/or treated by methods and compositions of the invention include cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malig melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia. Moreover, miRNA can be evaluated in precancers, such as metaplasia, dysplasia, and hyperplasia.

It is specifically contemplated that the invention can be used to evaluate or diagnose differences between stages of disease, such as between pre-cancer and cancer, or between a primary tumor and a metastasized tumor.

The efficacy of different therapeutic drugs is altered by miRNAs according to the present invention. Such therapeutic drugs include, but are not limited to, chemotherapeutic drugs. A "chemotherapeutic agent" is used to connote a compound or composition that is administered in the treatment of cancer. These agents or drugs are categorized by their mode of activity within a cell, for example, whether and at what stage they affect the cell cycle. Alternatively, an agent may be characterized based on its ability to directly cross-link DNA, to intercalate into DNA, or to induce chromosomal and mitotic aberrations by affecting nucleic acid synthesis. Most chemotherapeutic agents fall into the following categories: alkylating agents, antimetabolites, antitumor antibiotics, mitotic inhibitors, and nitrosoureas.

Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e.g.,* calicheamicin, especially calicheamicin gammalI and calicheamicin omegaI1; dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, paclitaxel and doxetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum coordination complexes such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate, exemestane, formestanie, fadrozole, vorozole, letrozole, and anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; ribozymes such as a VEGF expression inhibitor and a HER2 expression inhibitor; vaccines such as gene therapy vaccines and pharmaceutically acceptable salts, acids or derivatives of any of the above. A list of U.S. FDA approved oncology drugs with their approved indications can be found on the World Wide Web. Moreover, it is contemplated that samples that have differences in the activity of certain pathways may also be compared. Such cellular pathways include but are not limited to the following: any adhesion or motility pathway including but not limited to those involving cyclic AMP, protein kinase A, G-protein couple receptors, adenylyl cyclase, L-selectin, E-selectin, PECAM, VCAM-1, α-actinin, paxillin, cadherins, AKT, integrin-α, integrin-β, RAF-1, ERK, PI-3 kinase, vinculin, matrix metalloproteinases, Rho GTPases, p85, trefoil factors, profilin, FAK, MAP kinase, Ras, caveolin, calpain-1, calpain-2, epidermal growth factor receptor, ICAM-1, ICAM-2, cofilin, actin, gelsolin, RhoA, RAC1, myosin light chain kinase, platelet-derived growth factor receptor or ezrin; any apoptosis pathway including but not limited to those involving AKT, Fas ligand, NF_KB, caspase-9, PI3 kinase, caspase-3, caspase-7, ICAD, CAD, EndoG, Granzyme B, Bad, Bax, Bid, Bak, APAF-1, cytochrome C, p53, ATM, Bcl-2, PARP, Chk1, Chk2, p21, c-Jun, p73, Rad51, Mdm2, Rad50, c-Abl, BRCA-1, perforin, caspase-4, caspase-8, caspase-6, caspase-1, caspase-2, caspase-10, Rho, Jun kinase, Jun kinase kinase, Rip2, lamin-A, lamin-B1, lamin-B2, Fas receptor, H_2O_2, Granzyme A, NADPH oxidase, HMG2, CD4, CD28, CD3, TRADD, IKK, FADD, GADD45, DR3 death receptor, DR4/5 death receptor, FLIPs, APO-3, GRB2, SHC, ERK, MEK, RAF-1, cyclic AMP, protein kinase A, E2F, retinoblastoma protein, Smac/Diablo, ACH receptor, 14-3-3, FAK, SODD, TNF receptor, RIP, cyclin-D1, PCNA, Bcl-XL, PIP2, PIP3, PTEN, ATM, Cdc2, protein kinase C, calcineurin, IKKα, IKKβ, IKKγ, SOS-1, c-FOS, Traf-1, Traf-2, $I_KB\beta$ or the proteasome; any cell activation pathway including but not limited to those involving protein kinase A, nitric oxide, caveolin-1, actin, calcium, protein kinase C, Cdc2, cyclin B, Cdc25, GRB2, SRC protein kinase, ADP-ribosylation factors (ARFs), phospholipase D, AKAP95, p68, Aurora B, CDK1, Eg7, histone H3, PKAc, CD80, PI3 kinase, WASP, Arp2, Arp3, p16, p34, p20, PP2A, angiotensin, angiotensin-converting enzyme, protease-activated receptor-1, protease-activated receptor-4, Ras, RAF-1, PLCβ, PLCγ, COX-1, G-protein-coupled receptors, phospholipase A2, IP3, SUMO1, SUMO 2/3, ubiquitin, Ran, Ran-GAP, Ran-GEF, p53, glucocorticoids, glucocorticoid receptor, components of the SWI/SNF complex, RanBP1, RanBP2, importins, exportins, RCC1, CD40, CD40 ligand, p38, IKKα, IKKβ, $NF_{K}B$, TRAF2, TRAF3, TRAF5, TRAF6, IL-4, IL-4 receptor, CDK5, AP-1 transcription factor, CD45, CD4, T cell receptors, MAP kinase, nerve growth factor, nerve growth factor receptor, c-Jun, c-Fos, Jun kinase, GRB2, SOS-1, ERK-1, ERK, JAK2, STAT4, IL-12, IL-12 receptor, nitric oxide synthase, TYK2, IFNγ, elastase, IL-8, epithelins, IL-2, IL-2 receptor, CD28, SMAD3, SMAD4, TGFβ or TGFβ receptor; any cell cycle regulation, signaling or differentiation pathway including but not limited to those involving TNFs, SRC protein kinase, Cdc2, cyclin B, Grb2, Sos-1, SHC, p68, Aurora kinases, protein kinase A, protein kinase C, Eg7, p53, cyclins, cyclin-dependent kinases, neural growth factor, epidermal growth factor, retinoblastoma protein, ATF-2, ATM, ATR, AKT, CHK1, CHK2, 14-3-3, WEE1, CDC25 CDC6, Origin Recognition Complex proteins, p15, p16, p27, p21, ABL, c-ABL, SMADs, ubiquitin, SUMO, heat shock proteins, Wnt, GSK-3, angiotensin, p73 any PPAR, TGFα, TGFβ, p300, MDM2, GADD45, Notch, cdc34, BRCA-1, BRCA-2, SKP1, the proteasome, CUL1, E2F, p107, steroid hormones, steroid hormone receptors, $I_{K}B\alpha$, $I_{K}B\beta$, Sin3A, heat shock proteins, Ras, Rho, ERKs, IKKs, PI3 kinase, Bcl-2, Bax, PCNA, MAP kinases, dynein, RhoA, PKAc, cyclin AMP, FAK, PIP2, PIP3, integrins, thrombopoietin, Fas, Fas ligand, PLK3, MEKs, JAKs, STATs, acetylcholine, paxillin calcineurin, p38, importins, exportins, Ran, Rad50, Rad51, DNA polymerase, RNA polymerase, Ran-GAP, Ran-GEF, NuMA, Tpx2, RCC1, Sonic Hedgehog, Crm1, Patched (Ptc-1), MPF, CaM kinases, tubulin, actin, kinetochore-associated proteins, centromere-binding proteins, telomerase, TERT, PP2A, c-MYC, insulin, T cell receptors, B cell receptors, CBP, IKB, $NF_{K}B$, RAC1, RAF1, EPO, diacylglycerol, c-Jun, c-Fos, Jun kinase, hypoxia-inducible factors, GATA4, β-catenin, α-catenin, calcium, arrestin, survivin, caspases, procaspases, CREB, CREM, cadherins, PECAMs, corticosteroids, colony-stimulating factors, calpains, adenylyl cyclase, growth factors, nitric oxide, transmembrane receptors, retinoids, G-proteins, ion channels, transcriptional activators, transcriptional coactivators, transcriptional repressors, interleukins, vitamins, interferons, transcriptional corepressors, the nuclear pore, nitrogen, toxins, proteolysis, or phosphorylation; or any metabolic pathway including but not limited to those involving the biosynthesis of amino acids, oxidation of fatty acids, biosynthesis of neurotransmitters and other cell signaling molecules, biosynthesis of polyamines, biosynthesis of lipids and sphingolipids, catabolism of amino acids and nutrients, nucleotide synthesis, eicosanoids, electron transport reactions, ER-associated degradation, glycolysis, fibrinolysis, formation of ketone bodies, formation of phagosomes, cholesterol metabolism, regulation of food intake, energy homeostasis, prothrombin activation, synthesis of lactose and other sugars, multi-drug resistance, biosynthesis of phosphatidylcholine, the proteasome, amyloid precursor protein, Rab GTPases, starch synthesis, glycosylation, synthesis of phoshoglycerides, vitamins, the citric acid cycle, IGF-1 receptor, the urea cycle, vesicular transport, or salvage pathways. It is further contemplated that nucleic acids molecules of the invention can be employed in diagnostic and therapeutic methods with respect to any of the above pathways or factors. Thus, in some embodiments of the invention, a synthetic miRNA, nonsynthetic nucleic acid, or miRNA inhibitor inhibits, eliminate, activates, induces, increases, or otherwise modulates one or more of the above pathways or factors is contemplated as part of methods of the invention. The nucleic acid can be used to diagnosis a disease or condition based on the relation of that miRNA to any of the pathways described above.

Phenotypic traits also include characteristics such as longevity, appearance (e.g., baldness, obesity), strength, speed, endurance, fertility, and susceptibility or receptivity to particular drugs or therapeutic treatments. Synthetic miRNAs or miRNA inhibitors that affect phenotypic traits may provide intervention points for therapeutic development.

H. Other Assays

In addition to the use of arrays and microarrays, it is contemplated that a number of difference assays could be employed to analyze miRNAs, their activities,a nd their effects. Such assays include, but are not limited to, RT-PCR, *in situ* hybridization, hybridization protection assay (HPA)(GenProbe), branched DNA (bDNA) assay (Chiron), rolling circle amplification (RCA), single molecule hybridization detection (US Genomics), Invader^{TM} assay (ThirdWave Technologies), and Bridge Litigation Assay^{TM} (Genaco). It is contemplated that such methods may be used in the context of arrays, as well as in the context of diagnostic assays.

III. Therapeutic and Diagnostic Applications

Synthetic miRNAs or miRNA inhibitors that affect phenotypic traits provide intervention points for therapeutic applications as well as diagnostic applications (by screening for the presence or absence of a particular miRNA). It is specifically contemplated that RNA molecules of the present invention can be used to treat any of the diseases or conditions discussed in the previous section. Moreover, any of the methods described above can also be employed with respect to therapeutic and diagnostic aspects of the invention. For example, methods with respect to detecting miRNAs or screening for them can also be employed in a diagnostic context.

In therapeutic applications, an effective amount of the synthetic miRNAs or miRNA inhibitors of the present invention is administered to a cell, which may or may not be in an animal. In some embodiments, a therapeutically effective amount of the synthetic miRNAs or miRNA inhibitors of the present invention is administered to an individual for the treatment of disease or condition. The term "effective amount" as used herein is defined as the amount of the molecules of the present invention that are necessary to result in the desired physiological change in the cell or tissue to which it is administered. The term "therapeutically effective amount" as used herein is defined as the amount of the molecules of the present invention that achieves a desired effect with respect to a disease or condition. A skilled artisan readily recognizes that in many cases the molecules may not provide a cure but may provide a partial benefit, such as alleviation or improvement of at least one symptom. In some embodiments, a physiological change having some benefit is also considered therapeutically beneficial. Thus, in some embodiments, an amount of molecules that provides a physiological change is considered an "effective amount" or a "therapeutically effective amount."

In some embodiments the molecule has a sequence that corresponds to the miRNA sequence from that particular animal, as opposed to from another animal. Thus, in some embodiments, a human sequence is utilized in the RNA molecules of the present invention.

A. Modes of Administration and Formulations

The nucleic acid molecules of the invention may be administered to a subject alone or in the form of a pharmaceutical composition for the treatment of a condition or disease. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the proteins into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For topical administration the proteins of the invention may be formulated as solutions, gels, ointments, creams, suspensions, *etc.* as are well-known in the art. Systemic formulations include those designed for administration by injection, *e.g.* subcutaneous, intravenous, intramuscular, intrathecal or intraperitoneal injection, as well as those designed for transdermal, transmucosal, inhalation, oral or pulmonary administration. For injection, the nucleic acids of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the nucleic acid molecules may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For oral administration, the nucleic acids can be readily formulated by combining the molecules with pharmaceutically acceptable carriers well known in the art. Such carriers enable the nucleic acids of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. For oral solid formulations such as, for example, powders, capsules and tablets, suitable excipients include fillers such as sugars, *e.g.* lactose, sucrose, mannitol and sorbitol; cellulose preparations such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP); granulating agents; and binding agents. If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. If desired, solid dosage forms may be sugar-coated or enteric-coated using standard techniques. For oral liquid preparations such as, for example, suspensions, elixirs and solutions, suitable carriers, excipients or diluents include water, glycols, oils, alcohols, *etc.* Additionally, flavoring agents, preservatives, coloring agents and the like may be added. For buccal administration, the molecules may take the form of tablets, lozenges, *etc.* formulated in conventional manner. For administration by inhalation, the molecules for use according to the present invention are conveniently delivered in the form of an aerosol spray from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the nucleic acids and a suitable powder base such as lactose or starch. The RNA molecules may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the molecules may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the molecules may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Alternatively, other pharmaceutical delivery systems may be employed. Liposomes and emulsions are well-known examples of delivery vehicles that may be used to deliver nucleic acids of the invention.

A nucleic acid of the invention may be administered in combination with a carrier or lipid to increase cellular uptake. For example, the oligonucleotide may be administered in combination with a cationic lipid. Examples of cationic lipids include, but are not limited to, lipofectin, DOTMA, DOPE, and DOTAP. The publication of WO0071096 describes different formulations, such as a DOTAP:cholesterol or cholesterol derivative formulation that can effectively be used for gene therapy. Other disclosures also discuss different lipid or liposomal formulations including nanoparticles and methods of administration; these include, but are not limited to, U.S. Patent Publication 20030203865, 20020150626, 20030032615, and 20040048787, to the extent they disclose formulations and other related aspects of administration and delivery of nucleic acids. Methods used for forming particles are also disclosed in U.S. Pat. Nos. 5,844,107, 5,877,302, 6,008,336, 6,077,835, 5,972,901, 6,200,801, and 5,972,900.

The nucleic acids may also be administered in combination with a cationic amine such as poly (L-lysine). Nucleic acids may also be conjugated to a chemical moiety, such as transferrin and cholesteryls. In addition, oligonucleotides may be targeted to certain organelles by linking specific chemical groups to the oligonucleotide. For example, linking the oligonucleotide to a suitable array of mannose residues will target the oligonucleotide to the liver.

Additionally, the molecules may be delivered using a sustained-release system, such as semipermeable matrices of solid polymers containing the therapeutic agent. Various of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the molecules for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the chimeric molecules, additional strategies for molecule stabilization may be employed.

Nucleic acids may be included in any of the above-described formulations as the free acids or bases or as pharmaceutically acceptable salts. Pharmaceutically acceptable salts are those salts that substantially retain the biologic activity of the free bases and which are prepared by reaction with inorganic acids. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free base forms.

Pharmaceutical compositions of the present invention comprise an effective amount of one or more synthetic miRNA molecules or miRNA inhibitors dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of an pharmaceutical composition that contains at least one chimeric polypeptide or additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990. Moreover, for animal (*e.g.*, human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The chimeric molecules may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. The present invention can be administered intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostaticaly, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctival, intravesicularlly, mucosally, intrapericardially, intraumbilically, intraocularally, orally, topically, locally, inhalation (*e.g.* aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, *via* a catheter, *via* a lavage, in cremes, in lipid compositions (*e.g.*, liposomes), or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990).

The actual dosage amount of a composition of the present invention administered to an animal patient can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, the an active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, *etc.*, can be administered, based on the numbers described above.

In any case, the composition may comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents, including but not limited to parabens (*e.g.*, methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

The molecules may be formulated into a composition in a free base, neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts, *e.g.*, those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine.

In embodiments where the composition is in a liquid form, a carrier can be a solvent or dispersion medium comprising but not limited to, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, liquid polyethylene glycol, *etc.*), lipids (*e.g.*, triglycerides, vegetable oils, liposomes) and combinations thereof. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin; by the maintenance of the required particle size by dispersion in carriers such as, for example liquid polyol or lipids; by the use of surfactants such as, for example hydroxypropylcellulose; or combinations thereof such methods. In many cases, it will be preferable to include isotonic agents, such as, for example, sugars, sodium chloride or combinations thereof.

In other embodiments, one may use eye drops, nasal solutions or sprays, aerosols or inhalants in the present invention. Such compositions are generally designed to be compatible with the target tissue type. In a non-limiting example, nasal solutions are usually aqueous solutions designed to be administered to the nasal passages in drops or sprays. Nasal solutions are prepared so that they are similar in many respects to nasal secretions, so that normal ciliary action is maintained. Thus, in preferred embodiments the aqueous nasal solutions usually are isotonic or slightly buffered to maintain a pH of about 5.5 to about 6.5. In addition, antimicrobial preservatives, similar to those used in ophthalmic preparations, drugs, or appropriate drug stabilizers, if required, may be included in the formulation. For example, various commercial nasal preparations are known and include drugs such as antibiotics or antihistamines.

In certain embodiments, the molecules are prepared for administration by such routes as oral ingestion. In these embodiments, the solid composition may comprise, for example, solutions, suspensions, emulsions, tablets, pills, capsules (*e.g.*, hard or soft shelled gelatin capsules), sustained release formulations, buccal compositions, troches, elixirs, suspensions, syrups, wafers, or combinations thereof. Oral compositions may be incorporated directly with the food of the diet. Preferred carriers for oral administration comprise inert diluents, assimilable edible carriers or combinations thereof. In other aspects of the invention, the oral composition may be prepared as a syrup or elixir. A syrup or elixir, and may comprise, for example, at least one active agent, a sweetening agent, a preservative, a flavoring agent, a dye, a preservative, or combinations thereof.

In certain preferred embodiments an oral composition may comprise one or more binders, excipients, disintegration agents, lubricants, flavoring agents, and combinations thereof. In certain embodiments, a composition may comprise one or more of the following: a binder, such as, for example, gum tragacanth, acacia, cornstarch, gelatin or combinations thereof; an excipient, such as, for example, dicalcium phosphate, mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate or combinations thereof; a disintegrating agent, such as, for example, corn starch, potato starch, alginic acid or combinations thereof; a lubricant, such as, for example, magnesium stearate; a sweetening agent, such as, for example, sucrose, lactose, saccharin or combinations thereof; a flavoring agent, such as, for example peppermint, oil of wintergreen, cherry flavoring, orange flavoring, *etc.*; or combinations thereof the foregoing. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, carriers such as a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both.

The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less that 0.5 ng/mg protein.

In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

Any embodiment discussed above with respect to delivery or transport to cells can also be employed with respect to implementing delivery of medicinal compounds discussed in this section.

B. Effective Dosages

The molecules of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the molecules of the invention, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. A therapeutically effective amount is an amount effective to ameliorate or prevent the symptoms, or prolong the survival of, the patient being treated. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_5 as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

Initial dosages can also be estimated from *in vivo* data, *e.g.*, animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

Dosage amount and interval may be adjusted individually to provide plasma levels of the molecules which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 5 mg/kg/day, preferably from about 0.5 to 1 mg/kg/day. Therapeutically effective serum levels may be achieved by administering multiple doses each day.

In cases of local administration or selective uptake, the effective local concentration of the proteins may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

The amount of molecules administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

The therapy may be repeated intermittently while symptoms detectable or even when they are not detectable. The therapy may be provided alone or in combination with other drugs or treatment (including surgery).

C. Toxicity

Preferably, a therapeutically effective dose of the molecules described herein will provide therapeutic benefit without causing substantial toxicity.

Toxicity of the molecules described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD50 (the dose lethal to 50% of the population) or the LD_{100} (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. Proteins which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of the proteins described herein lies preferably within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See, *e.g.*, Fingl *et al.*, 1975, In: The Pharmacological Basis of Therapeutics, Ch.1, p.1).

D. Pendant Groups

A "pendant group" may be attached or conjugated to the nucleic acid. Pendant groups may increase cellular uptake of the nucleic acid. Pendant groups can be linked to any portion of the nucleic acid but are commonly linked to the end(s) of the oligonucleotide chain. Examples of pendant groups include, but are not limited to: acridine derivatives (i.e. 2-methoxy-6-chloro-9-aminoacridine); cross-linkers such as psoralen derivatives, azidophenacyl, proflavin, and azidoproflavin; artificial endonucleases; metal complexes such as EDTA-Fe(II), o-phenanthroline-Cu(I), and porphyrin-Fe(II); alkylating moieties; nucleases such as amino-1-hexanolstaphylococcal nuclease and alkaline phosphatase; terminal transferases; abzymes; cholesteryl moieties; lipophilic carriers; peptide conjugates; long chain alcohols; phosphate esters; amino; mercapto groups; radioactive markers; nonradioactive markers such as dyes; and polylysine or other polyamines. In one example, the nucleic acid is conjugated to a carbohydrate, sulfated carbohydrate, or glycan.

IV. Kits

Any of the compositions described herein may be comprised in a kit. In a non-limiting example, individual synthetic miRNAs are included in a kit. The kit may further include one or more negative control synthetic miRNAs that can be used to control for the effects of synthetic miRNA delivery. The kit may further include water and hybridization buffer to facilitate hybridization of the two strands of the synthetic miRNAs. The kit may also include one or more transfection reagent(s) to facilitate delivery of the synthetic miRNA to cells.

In another non-limiting example, multiple synthetic miRNAs and/or multiple miRNA inhibitors are included in a kit. The kit may further include one or more negative control synthetic miRNAs and/or miRNA inhibitors that can be used to control for the effects of synthetic miRNA and/or miRNA inhibitor delivery. The kit may also include one or more transfection reagents to facilitate delivery into cells.

The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there is more than one component in the kit (labeling reagent and label may be packaged together), the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present invention also will typically include a means for containing the nucleic acids, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred.

However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

The container means will generally include at least one vial, test tube, flask, bottle, syringe and/or other container means, into which the nucleic acid formulations are placed, preferably, suitably allocated. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent.

The kits of the present invention will also typically include a means for containing the vials in close confinement for commercial sale, such as, *e.g.*, injection and/or blow-molded plastic containers into which the desired vials are retained.

Such kits may also include components that preserve or maintain the miRNA or that protect against its degradation. Such components may be RNAse-free or protect against RNAses. Such kits generally will comprise, in suitable means, distinct containers for each individual reagent or solution.

A kit will also include instructions for employing the kit components as well the use of any other reagent not included in the kit. Instructions may include variations that can be implemented.

Kits of the invention may also include one or more of the following: synthetic miRNA, nonsynthetic miRNA, library of synthetic miRNAs, library of miRNA inhibitors, library of nonsynthetic miRNA, combination library of synthetic miRNA, miRNA inhibitors, and/or nonsynthetic miRNAs, negative control synthetic miRNA, negative control miRNA inhibitor, negative control nonsynthetic miRNA, nuclease-free water; RNase-free containers, such as 1.5 ml tubes; hybridization buffer; and transfection reagent(s).

It is contemplated that such reagents are embodiments of kits of the invention. Such kits, however, are not limited to the particular items identified above and may include any reagent used for the manipulation or characterization of miRNA.

Examples

Unless otherwise designated, catalog numbers refer to products available by that number from Ambion, Inc.®, The RNA Company.

EXAMPLE 1:

Assay for measuring activity of precursor miRNAs (reporter)

A series of luciferase reporter vectors was created to measure the activities of synthetic miRNAs in cells. The reporter vectors were based on plasmids that had been used to monitor the activity of endogenous miRNAs (Tuschl paper). Briefly, a mammalian expression vector with the luciferase gene under the control of the CMV early promoter was created. Down-stream of the luciferase coding sequence, in the 3′

UTR of the gene, sequences complementary to mature miR-1-2, miR-10, miR-124, miR-19a, and miR-130 were added. The reporter vectors were co-transfected into HeLa cells along with synthetic miRNAs designed to introduce one of the five miRNAs listed above. The transfections involved mixing 200 ng of reporter vector with 0.3, 1, and 3 pmoles of each corresponding synthetic miRNA. The reporter/miRNA mixture was mixed with 0.3 μl of Lipofectamine 2000™ (Invitrogen) and incubated for 5-15 minutes. Approximately 8,000 cells were added to each miRNA/reporter/transfection reagent complex in individual wells of a 96-well plate. HeLa cells were grown in D-MEM (GIBCO) supplemented with 10% fetal bovine serum (GIBCO) at 37°C and 5% CO_2. 24-48 hrs post transfection, the cells were harvested and assayed using the Luciferase assay as described by the manufacturer (Promega). The level of luciferase expression in the cell populations was compared to cells transfected with the same reporter but a synthetic miRNA with a sequence that does not correspond to the vector. This non-targeting miRNA was referred to as the negative control miRNA.

Final analysis of the synthetic miRNA designs involved measuring the activity of both the active and complementary strands of our synthetic miRNAs. For these studies, reporter vectors with luciferase 3′ UTR sequences were created that included regions complementary to both the active and the complementary strands of our synthetic miR-33 and let-7b miRNA designs. When co-transfected with malfunctioning synthetic miRNAs, the reporters with a sequence targeted by the complementary strand exhibit reduced luciferase expression because the complementary strand of the synthetic miRNAs are entering the miRNA pathway in addition to or even instead of the active strand that is desired. For these experiments, the co-transfection and reporter analysis protocols are identical to what is described above.

EXAMPLE 2:

Assay for measuring activity of precursor miRNAs (Endogenous gene)

While the luciferase reporter constructs were extremely valuable in evaluating the synthetic miRNA designs, it was important to verify the findings of the reporter constructs by measuring the effects of the synthetic miRNAs on endogenous gene targets. For these studies, the expression of RAS and MYC in cells transfected with let-7 miRNAs was chosen for monitoring. Both RAS and MYC are down-regulated by the various members of the let-7 family in humans and *C. elegans* (publication pending). Using a microarray system specific to miRNAs, the inventors have found that HepG2 cells express undetectable levels of let-7. To test the activities of our various designs of our synthetic miRNAs, synthetic let-7 miRNAs were created and used to transfect HepG2 cells in 24-well plates using siPORT NeoFX (Ambion) according to the manufacturer's suggestions. Three days post-transfection, the cells were fixed with 4% paraformaldehyde, stained with DAPI to localize cell nuclei, and stained with FITC-conjugated antibodies specific to MYC or RAS (US Biological) according to the manufacturer's suggestions. The relative reduction in target protein expression in synthetic let-7 transfected cells was determined by comparing the staining intensity of MYC and RAS to cells transfected with a negative control miRNA using MetaMorph software.

To ensure that the results of our let-7 assays could be verified by additional miRNA interactions that are observed naturally in cells, we created assays for two additional miRNAs with verified targets. In the first, a real-time PCR™ assay was developed to measure the level of the HOXB8 mRNA in cells transfected with synthetic miR-196. It has been shown that miR-196 induces degradation of the HOXB8 mRNA in cells. When transfected into cultured cells using siPORT NeoFX according to the manufacturer's instructions, effective miR-196 synthetic miRNA designs reduce the levels of the HOXB8 mRNA.

To monitor the effectiveness of synthetic miR-1-2 miRNAs, a reporter vector was created wherein the 3′ UTR of the G6PD gene was placed immediately downstream of the luciferase coding region. An interaction between miR-1-2 and the

G6PD 3′ UTR has been published (Lewis, 2003). Synthetic miR-1-2 designs were co-transfected with the reporter vector and assayed as described in Example 1.

EXAMPLE 3

Effectiveness of partially complementary miRNAs

Three general sequence designs were compared for miRNA activity. The first, referred to as the "miRNA design," featured an active strand identical to the mature miRNA found in animals and a complementary strand that was identical to the hairpin sequence that is predicted to exist in cells during the processing of the miRNA prior to activation of the miRNA (see below). The second design, referred to as the "mismatch design," was a hybrid of the same active strand as above with a complementary strand with a di-nucleotide, 3′ overhang and two mismatches in the final five nucleotides that preceded the 3′ overhang (see below). The third design, referred to as the "siRNA design," comprised the same active strand as above hybridized to a second RNA that was fully complementary except that it left 3′ di-nucleotide overhangs at either end of the double-stranded molecule (two polynucleotides) (see below). The examples below involve or correspond to human miRNAs.

miR-1-2

mature miR-1-2 sequence- UGGAAUGUAAAGAAGUAUGUA (53-73 of SEQ ID NO:1)

miRNA design = CAUACUUCUUUAUAUGCCCAUA (SEQ ID NO:594) + UGGAAUGUAAAGAAGUAUGUA (SEQ ID NO:595)

mismatch design = CAUACUUCUUUACAUUCUGTT (SEQ ID NO:596) + UGGAAUGUAAAGAAGUAUGUA (SEQ ID NO:597)

siRNA design = CAUACUUCUUUACAUUCCATT (SEQ ID NO:598) + UGGAAUGUAAAGAAGUAUGUA (SEQ ID NO:599)

mir-124a-1

mature miR-124 sequence- UUAAGGCACGCGGUGAAUGCCA (52-73 of SEQ ID NO:80)

miRNAdesign = GUGUUCACAGCGGACCUUGAUU (SEQ ID NO:600) + UUAAGGCACGCGGUGAAUGCCA (SEQ ID NO:601) mismatch design = GCAUUCACCGCGUGCCUUGGTT (SEQ ID NO:602) + UUAAGGCACGCGGUGAAUGCCA (SEQ ID NO:603)

siRNA design = GCAUUCACCGCGUGCCUUAATT (SEQ ID NO:604) + UUAAGGCACGCGGUGAAUGCCA (SEQ ID NO:605)

miR-130a

mature miR-130 sequence – CAGUGCAAUGUUAAAAGGGC (55-74 of SEQ ID NO:91)

miRNA design = UCUUUUCACAUUGUGCUAC (SEQ ID NO:606) + CAGUGCAAUGUUAAAAGGGC (SEQ ID NO:607)

mismatch design = UAUUUUAACAUUGCACUGTT (SEQ ID NO:608) + CAGUGCAAUGUUAAAAGGGC (SEQ ID NO:609)

siRNA design = CCUUUUAACAUUGCACUGTT (SEQ ID NO:610) + CAGUGCAAUGUUAAAAGGGC (SEQ ID NO:611)

miR-19a

mature miR-19a sequence – UGUGCAAAUCUAUGCAAAACUGA (49-71 of SEQ ID NO:28)

miRNA design = AGUUUUGCAUAGUUGCACUA (SEQ ID NO:612) + UGUGCAAAUCUAUGCAAAACUGA (SEQ ID NO:613)

mismatch design = ACAUUUGCAUAGAUUUGCACATT (SEQ ID NO:614) + UGUGCAAAUCUAUGCAAAACUGA (SEQ ID NO:615)

siRNA design = AGUUUUGCAUAGAUUUGCACATT (SEQ ID NO:616)+ UGUGCAAAUCUAUGCAAAACUGA (SEQ ID NO:617)

mmu-miR-10a-1 (mouse)

mature miR-10 sequence – UACCCUGUAGAUCCGAAUUUGUG (22-44 of SEQ ID NO:212)

miRNA design = CAAAUUCGUAUCUAGGGGAAUA (SEQ ID NO:618) + UACCCUGUAGAUCCGAAUUUGUG (SEQ ID NO:619)

mismatch design = AGAAUUCGGAUCUACAGGGUATT (SEQ ID NO:620) + UACCCUGUAGAUCCGAAUUUGUG (SEQ ID NO:621)

siRNA design = CAAAUUCGGAUCUACAGGGUATT (SEQ ID NO:622) + UACCCUGUAGAUCCGAAUUUGUG (SEQ ID NO:623)

miR-33

mature miR-33 sequence – GUGCAUUGUAGUUGCAUUG (6-24 of SEQ ID NO:57)

miRNA = AUGUUUCCACAGUGCAUCA (SEQ ID NO:624) + GUGCAUUGUAGUUGCAUUG (SEQ ID NO:625)

mismatch design = GUCCAACUACAAUGCACTT (SEQ ID NO:626) + GUGCAUUGUAGUUGCAUUG (SEQ ID NO:627)

siRNA design = AUGCAACUACAAUGCACTT (SEQ ID NO:628) + GUGCAUUGUAGUUGCAUUG (SEQ ID NO:629)

let-7b

mature let-7b sequence – UGAGGUAGUAGGUUGUGUGGUU (6-27 of SEQ ID NO:6)

miRNA design = CUAUACAACCUACUGCCUUCC (SEQ ID NO:630) + UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:631)

mismatch design = CCACACAACCUACUAUCUUATT (SEQ ID NO:632) + UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:633)

siRNA design = CCACACAACCUACUACCUCATT (SEQ ID NO:634) + UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:635)

miR-196-2

mature miR-196 sequence – UAGGUAGUUUCAUGUUGUUGG (7-27 of SEQ ID NO:143)

siRNA design = AACAACAUGAAACUACCUATT (SEQ ID NO:636) + UAGGUAGUUUCAUGUUGUUGG (SEQ ID NO:637)

miRNA design = CAAAUUCGUAUCUAGGGGAAUA (SEQ ID NO:638) + UAGGUAGUUUCAUGUUGUUGG (SEQ ID NO:639)

mismatch design = AAUAACAUGAAACUACCUATT (SEQ ID NO:640) + UAGGUAGUUUCAUGUUGUUGG (SEQ ID NO:641)

The assorted mir-1-2, mmu-miR-10a-1, miR-19a, mir-124a-1, and mir-130a synthetic miRNAs were tested for their capacity to reduce the expression of the reporter gene in vectors with appropriate miRNA target sites using the assay described in Example 1.

All three designs were similarly capable of down-regulating the appropriate reporter vectors.

To assess whether there were differences between the various miRNA designs ini their ability to affect the expression of endogenous genes, the following cells were transfected: HepG2 cells with three designs of the let-7 synthetic miRNAs, A549 with three designs of the miR-196 synthetic miRNAs, and HeLa with the G6PD reporter vector and three designs of the miR-1-2 synthetic miRNA. As with the reporter vectors, all three synthetic miRNA designs proved capable of reducing the expression of the target genes, though it is notable that the siRNA design performed most poorly.

As a final comparison of the three synthetic miRNA designs, synthetic miRNAs were co-transfected with reporter vectors that included target sites for the complementary strands of the synthetic miRNAs according to the procedure described in Example 1. In this assay, it was apparent that the siRNA design significantly affected the reporter vectors, indicating that the wrong strand of the miRNA was entering the miRNA pathway (FIG. 3). Because the complementary strand might impact the expression of genes that are not natural targets of the miRNA that is being studied, the siRNA design is inappropriate for effective synthetic miRNAs.

EXAMPLE 4:

Effectiveness of chemically 5′ end-modified synthetic miRNAs

Although the siRNA design proved problematic in that it exhibited a high rate of complementary strand uptake by the miRNA pathway, it did have the advantage that it was easy to hybridize and easy to deliver to cells. For these reasons, ways to overcome the problems with complementary strand uptake were explored. The siRNA design was used to test the effects of chemical modifications at the 5′ ends of the synthetic miRNAs. For these studies, several different complementary strands were synthesized with unique 5′ ends. One featured four deoxyribose nucleotides at the 5′ end; one was a combination of four deoxyribose nucleotides at the 5′ end and a 5′ NH_2; one had a 5′ NH_2; one had a 5′ $NHCOCH_3$ (see below).

miR-33

mature miR-33 sequence – GUGCAUUGUAGUUGCAUUG (6-24 of SEQ ID NO:57) siRNA design = AUGCAACUACAAUGCACTT (SEQ ID NO:642) + GUGCAUUGUAGUUGCAUUG (SEQ ID NO:643)

5′ amino design = (NH_2)AUGCAACUACAAUGCACTT (SEQ ID NO:644) + GUGCAUUGUAGUUGCAUUG (SEQ ID NO:645)

5′ acetyl design = (CH_3OCNH)AUGCAACUACAAUGCACTT (SEQ ID NO:646) + GUGCAUUGUAGUUGCAUUG (SEQ ID NO:647)

5′ DNA design = dAdUdGdCAACUACAAUGCACTT (SEQ ID NO:648) + GUGCAUUGUAGUUGCAUUG (SEQ ID NO:649)

5′ amino DNA design = (NH_2)dAdUdGdCAACUACAAUGCACTT (SEQ ID NO:650) + GUGCAUUGUAGUUGCAUUG (SEQ ID NO:651)

let-7b

mature let-7b sequence – UGAGGUAGUAGGUUGUGUGGUU (6-27 of SEQ ID NO:6)

siRNA design = CCACACAACCUACUACCUCATT (SEQ ID NO:652) + UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:653)

5′ amino design = NH_2CCACACAACCUACUACCUCATT (SEQ ID NO:654) + UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:655)

5′ DNA design = dCdCdAdCACAACCUACUACCUCATT (SEQ ID NO:656) + UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:657)

5′ amino DNA design = NH_{2d}CdCdAdCACAACCUACUACCUCATT (SEQ ID NO:658) + UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:659)

miR-1-2

mature miR-1-2 sequence- UGGAAUGUAAAGAAGUAUGUA (53-73 of SEQ ID NO:1)

siRNA design = CAUACUUCUUUACAUUCCATT (SEQ ID NO:660) + UGGAAUGUAAAGAAGUAUGUA (SEQ ID NO:661)

5′ amino design = NH_2CAUACUUCUUUACAUUCCATT (SEQ ID NO:662) + UGGAAUGUAAAGAAGUAUGUA (SEQ ID NO:663)

miR-124a-1

mature miR-124 sequence- UUAAGGCACGCGGUGAAUGCCA (52-73 of SEQ ID NO:80)

siRNA design = GCAUUCACCGCGUGCCUUAATT (SEQ ID NO:664) + UUAAGGCACGCGGUGAAUGCCA (SEQ ID NO:665)

5′ amino design = NH_2GCAUUCACCGCGUGCCUUAATT (SEQ ID NO:666) + UUAAGGCACGCGGUGAAUGCCA (SEQ ID NO:667)

miR-130a

mature miR-130 sequence – CAGUGCAAUGUUAAAAGGGC (55-74 of SEQ ID NO:91)

siRNA design = CCUUUUAACAUUGCACUGTT (SEQ ID NO:668) + CAGUGCAAUGUUAAAAGGGC (SEQ ID NO:669)

5′ amino design = NH_2 CCUUUUAACAUUGCACUGTT (SEQ ID NO:670) + CAGUGCAAUGUUAAAAGGGC (SEQ ID NO:671)

miR-10a-1

mature miR-10 sequence – UACCCUGUAGAUCCGAAUUUGUG (22-44 of SEQ ID NO:212)

siRNA design = CAAAUUCGGAUCUACAGGGUATT (SEQ ID NO:672) + UACCCUGUAGAUCCGAAUUUGUG (SEQ ID NO:673)

5′amino design = NH_2CAAAUUCGGAUCUACAGGGUATT (SEQ ID NO:674) + UACCCUGUAGAUCCGAAUUUGUG (SEQ ID NO:675)

The miR-33 and let-7b synthetic miRNAs were co-transfected into HeLa and HepG2 cells, respectively, with reporter vectors bearing target sites for the active and complementary strands of miR-33 and let-7b as described in Example 1. Luciferase expression from the active and complementary strand-specific reporter vectors was measured according to the manufacturer's (Promega) protocol. As shown in FIG. 3, the synthetic miRNA designs with the 5′ NH_2 and 5′ $NHCOCH_3$ provided higher active strand activity and significantly reduced complementary strand activity relative to the unmodified, synthetic miRNAs. This is ideal for synthetic miRNAs since the effects seen following transfection will be specific to the activity of the active strand of the synthetic miRNA. Furthermore, the high efficacy of the 5′ modified designs will allow lower concentrations to be used for transfections and reduce toxicity that is often observed when transfecting cells with higher amounts of nucleic acid.

To confirm that the 5′ amino modification is superior to the standard siRNA design for a broad set of synthetic miRNAs, the effectiveness of both synthetic miRNA designs was measured in cells co-transfected with reporter vectors with miRNA target sites. As seen in FIG. 4, the 5′ NH_2 is reproducibly superior to the unmodified siRNA design.

EXAMPLE 5:

Effectiveness of chemically internally modified synthetic miRNAs

The siRNA design was also used to test the effects of chemical modifications at internal domains within the complementary strand. For these studies, 2'O-Me modifications were placed at various locations along the length of the complementary strand. Below is provided an example of a series of synthetic miRNAs with chemically modified complementary strands.

miRNA Strand - 5'-UAU ACA AGA GAU GAA AUC CUC-3' (SEQ ID NO:676)
Complementary Strands -
Position 1 - 5'-**GGA** UUU CAU CUC UUG UAU AUt-3'
Position 2 - 5'-GGA **UUU** CAU CUC UUG UAU AUt-3'
Position 3 - 5'-GGA UUU **CAU** CUC UUG UAU AUt-3'
Position 4 - 5'-GGA UUU CAU **CUC** UUG UAU AUt-3'
Position 5 - 5'-GGA UUU CAU CUC **UUG** UAU AUt-3'
Position 6 - 5'-GGA UUU CAU CUC UUG **UAU** AUt-3'
Note - Positions that are 2'-O-Me are denoted in bold.

Synthetic miRNAs with the designs described above were tested for miRNA and complementary strand activity. Interestingly, complementary strand modifications at positions 1 and 5 significantly reduced complementary strand activity without altering the activity of the miRNA strand (FIG. 3).

EXAMPLE 6:

Synthetic miRNA library screen for miRNAs that influence cell proliferation

A hallmark of cancer is uncontrolled cell proliferation; cell proliferation assays are commonly used by researchers to study the influence of genes in oncogenesis. A cell proliferation assay was used in conjunction with the miRNA inhibitor library to identify miRNAs that influence cell proliferation.

The inventors transfected HeLa cells in triplicate with fifteen different synthetic miRNAs using siPORT NeoFX (Ambion) according to the manufacturer's instructions (FIG. 6). Transfected HeLa cells were analyzed using Alamar Blue (BioSource International, Inc., CA) at 24 hr intervals. Alamar Blue is a compound, that when reduced by cellular metabolism, changes from a non-fluorescent blue color to a fluorescent red form that is easily quantified. The amount of Alamar Blue reduced is directly proportional to the cell number, providing a rapid method for assessing cell proliferation. To perform the assay, the Alamar Blue reagent was added into the tissue culture media at a 10% final concentration. The mixture was incubated for 3-6 hr in growth conditions after which fluorescence was quantified using a Spectra Max™ GeminiXS™ (Molecular Devices, Sunnyvale, CA). Cells transfected with synthetic miR-124 and miR-106 exhibited significantly lower proliferation than negative control-transfected samples, as well as smaples transfected with the other synthetic miRNAs.

EXAMPLE 7:

MiRNA inhibitor library screen for miRNAs that influence cell proliferation

A hallmark of cancer is uncontrolled cell proliferation. Cell proliferation assays are commonly used by researchers to study the influence of genes in oncogenesis. A cell proliferation assay was used in conjunction with our miRNA inhibitor library to identify miRNAs that influence cell proliferation.

Cells were transfected with a library of over 90 miRNA inhibitors to identify miRNAs that are involved in cell growth. HeLa cells (8000 cells/well of 96 well plate) were transfected in triplicate with 5 pmoles of miRNA inhibitors using siPORT™ *NeoFX*™ (Ambion). The media was changed 24 hrs after transfection. 72 hours post-transfection, we fixed cells with 4% paraformaldehyde, permiabilized with 0.1% TritonX 100, and stained with propidium iodide to look at total cell number. The plates were scaned using the TTP labtech Acumen Explorer. Cell number was plotted relative to cells transfected with a negative control miRNA inhibitor (FIG. 7). The red horizontal bars bracket normal variation in cell proliferation (20% variation). Insets: Specific miRNA inhibitors that either increased cell proliferation (left arrow) or did not affect cell proliferation (right arrow) were used in a second round of screening. HeLa cells were transfected with these miRNA inhibitors and cells were fixed and stained with anti b-actin antibody and DAPI to visualize cell morphology changes in response to specific miRNA function. Cells transfected with the miRNA inhibitor that increased cell proliferation show marked alteration in cell morphology (left inset) vs. normal morphology (right inset).

A group of nine miRNA inhibitors were identified that caused significant decreases (miR 31, 150, 187, 125a, 190, 191, 193, 204 and 218) in cell growth and two miRNA inhibitors that caused a significant increase (miR 24 and miR 21) in cell growth following transfection into HeLa cells (Table 4). MiRNA-31 inhibition also caused a distinct cellular morphology. A relative cut off of 20% above and below 100% was chosen as genes that were considered significantly changed. These results demonstrate the ability of individual human miRNAs to regulate important cellular processes. Furthermore, the diversity of the observed effects demonstrates the potential complexity of cellular outcomes of miRNA-mediated regulation of gene expression.

Table 4. MiRNAs that affect cell proliferation

miRNA	Relative Impact on Cell Proliferation
miR-31	Up regulation
miR-150	Up regulation
miR-187	Up regulation
miR-125a	Up regulation
miR-190	Up regulation
miR-191	Up regulation
miR-193	Up regulation
miR-204	Up regulation

miR218	Up regulation
miR-21	Down regulation
miR-24	Down regulation

EXAMPLE 8:

Synthetic miRNA library screen for miRNAs that influence apoptosis

Many diseases including cancer are characterized by an inability to institute programmed cell death, or apoptosis. A caspase 3/7 activity assay was used in conjunction with a library of synthetic miRNAs to identify miRNAs that are involved in regulating apoptosis.

A library of eighteen synthetic miRNAs was used to transfect A549 cells (8000 cells/well of 96 well plate) in triplicate using siPORT™ *NeoFX*™ (Ambion). Media was changed after 24 hrs and cells were visually inspected under a microscope to qualitatively inspect cell death 72 hours after transfection. The cells were measured for apoptosis by measuring caspase 3 activity as follows: 1) Cells were washed once with PBS and frozen at –80°C. 2) Cells were lysed by adding 40 µl of cold lysis buffer (50 mM HEPES pH 7.2, 40 mM NaCl , 0.5% NP40, 0.5 mM EDTA) to the wells and incubated for 20 min at 4°C. 3) Add 160 µl ICE buffer (50 mM HEPES pH 7.4, 0.1% CHAPS, 0.1 mM EDTA, 10% sucrose) + 5 mM DTT containing 20 µM DEVDafc substrate. 4) Measure fluorescence increase in one hour at 400 ex, 505 em.

Cells transfected with miR-1-2 and miR-33 synthetic miRNAs exhibited reduced caspase 3/7 activity and cells transfected with miR-20 exhibited much higher levels of apoptosis. These three miRNAs likely regulate genes that are involved in controlling apoptosis.

EXAMPLE 9:

Screen for miRNAs that influence cell viability

miRNA inhibitors were also used to identify miRNAs that influence cell viability. A library of over 90 miRNA inhibitors was used to transfect A549 cells (8000 cells/well of 96 well plate) in triplicate using siPORT™ *NeoFX*™ (Ambion). Media was changed after 24 hrs and cells were visually inspected under a microscope to qualitatively inspect cell death 72 hours after transfection. Cells were trypsinized and stained with ViaCount Flex Reagent, which distinguishes between viable and non-viable cells based on permeability of the DNA binding dyes in the reagent. Cells were analyzed using the Guava PCA-96 (Personal Cell Analysis).

Twenty-one miRNA inhibitors induced a significantly different ratio of live to dead cells than did the negative control miRNA inhibitor (FIG 8). Twelve reduced cell viability and nine increased cell viability (Table 5). Interestingly, there was littleoverlap in the miRNAs that affected cell viability in A549 cells and those that affected cell proliferation in HeLa cells, suggesting that different cells respond differently to have reduced miRNA activities or cell viability and cell proliferation are not affected by the same cellular pathways.

Table 5. MiRNAs that affect cell viability

miRNA	Relative Impact on Cell Viability
miR-7	Down
miR-19a	Down
miR-23	Down
miR-24	Down
miR-27a	Down
miR-31	Down
miR-32	Down
miR-134	Down
miR-140	Down
miR-150	Down
miR-192	Down
miR-193	Down
miR-107	Up
miR-133	Up
miR-137	Up
miR-152	Up
miR-155	Up
miR-181a	Up

miR-191	Up
miR-203	Up
miR-215	Up

EXAMPLE 10:

Screen for miRNAs That Influence Apoptosis

Apoptosis is a natural cellular process that helps control cancer by inducing death in cells with oncogenic potential. Many oncogenes function by altering induction of apoptosis. To identify miRNAs that participate in apoptosis, an apoptosis assay was used with the miRNA inhibitor library.

Using a library of over 90 miRNA inhibitors, we screened for miRNAs that affect apoptosis. HeLa cells (8000 cells/well of 96 well plate) were transfected in triplicate with miRNA inhibitors (5 pmoles) using Ambion siPORT™ *NeoFX*™. The media was changed 24 hrs after transfection and processed cells 72 hours after transfection. The cells were measured for apoptosis by measuring caspase 3 activity as follows: 1) Cells were washed once with PBS and frozen at –80°C. 2) Cells were lysed by adding 40 μl of cold lysis buffer (50 mM HEPES pH 7.2, 40 mM NaCl , 0.5% NP40, 0.5 mM EDTA) to the wells and incubated for 20 min at 4°C. 3) Add 160 μl ICE buffer (50 mM HEPES pH 7.4, 0.1% CHAPS, 0.1 mM EDTA, 10% sucrose) + 5 mM DTT containing 20 μM DEVDafc™ substrate. 4) Measure fluorescence increase in one hour at 400 ex, 505 em.

Samples were also analyzed for cell number using a general esterase assay to normalize the caspase 3 results. FDA substrate (0.4 mg/ml fluorescein diacetate (FDA) in acetonitrile) was diluted 1:19 into dilution buffer (40 mM TrisCl pH 7.5, 20 mM NaCl, 0.5% NP-40, 0.02 mg/ml final conc). 40 μl buffer (40 mM TrisCl pH 7.5, 0.5% NP-40) was added to each sample well. Samples were incubated 10 min on ice. 160 μl of diluted FDA substrate was added to each well. Fluorescence was measured for 30 min at 37°C (ex = 488, em = 529). The slope of fluorescence increase over time is a function of the cell number in the plate.

Normalized screening data are displayed in FIG. 9. miRNAs that affect apoptosis are listed in Table 6.

Table 6. MiRNAs that affect apoptosis

miRNA	Relative Impact on Cell Proliferation
miR-31	Down
miR-214	Down
miR-7	Up
miR-1-2	Up
miR-148	Up
miR-195	Up
miR-196	Up
miR-199a	Up
miR-204	Up
miR-210	Up
miR-211	Up
miR-212	Up
miR-215	Up
miR-216	Up
miR-218	Up
miR-296	Up
miR-321	Up

EXAMPLE 11:

Expression Analyses Using Synthetic RNAs

In addition to using phenotypic assays to identify miRNAs that influence gross cellular processes or cellular pathways, collections of synthetic miRNAs and/or miRNA inhibitors can be used to identify miRNAs that directly regulate the expression of a gene. A plasmid was created that had a luciferase gene immediately upstream of the 3'UTR of the G6PD gene. A549 cells were co-transfected with the reporter vector and eighteen different synthetic miRNAs. 24 hours post-transfection, luciferase activity in the various cell populations was measured. Interestingly, the miR-1-2 significantly reduced the expression of the luciferase/G6PD gene, indicating that this family of miRNAs regulates the expression of the G6PD gene. Similar experiments can be used to identify miRNAs that regulate the expression of such important genes as p53, BRCA1 and BRCA2, RAS, MYC, BCL-2, and others.

EXAMPLE 12:

Oncogeneic miRNAs—Differential Expression and Cancer Regulation

As noted in previous examples, a number of miRNAs have been identified that are differentially expressed between tumor and normal adjacent tissue samples from the same cancer patients. Interestingly, there is significant overlap in the miRNAs that are differentially expressed between different cancers, suggesting there is a core set of miRNAs that influence cellular processes that when altered, lead to cancer. The following describes experiments aimed at developing a link between miRNA mis-regulation and cancer.

miRNA Expression in Lung Cancer

Twenty-two tumor and normal adjacent tissue (NAT) samples from lung cancer patients were analyzed using the miRNA array system described above. The arrays were analyzed and the relative expression of each miRNA was compared between the tumor and normal adjacent tissues from each patient. The various miRNAs were clustered based on their relative expression in tumors across different patients (FIG. 14). Six miRNAs (miR-126, 30a, 143, 145, 188, and 331) were expressed at significantly lower levels in the tumors of more than 70% of the patients. Two miRNAs (miR-21 and 200b) were expressed at significantly higher levels in the tumors of more than 70% of the patients. The differential expression of a number of these miRNAs was verified by Northern analysis (FIG. 15).

miRNA Expression in Colon Cancer

Twenty-five tumor and NAT samples from colon cancer patients were analyzed using our miRNA array process. Like the lung cancer comparisons, the various miRNAs were clustered based on their relative expression in tumors across the different colon cancer patients (FIG. 14). Five miRNAs (miR-143, 145, 195, 130a, and miR-331) were expressed at significantly lower levels in the tumors of more than 70% of the patients. Five miRNAs (miR-223, 21, 31, 17, and 106) were expressed at significantly higher levels in the tumors of more than 70% of the patients.

miRNAs as Cancer Markers

It is interesting that eight different miRNAs were differentially expressed between the tumor and normal adjacent samples for most of the lung and colon patient samples that we analyzed (FIG. 16). These same miRNAs were also found to be differentially expressed in the breast, thymus, bladder, pancreatic, and prostate cancer patients that we analyzed, suggesting that these miRNAs might control cellular processes that when altered lead to cancer.

miRNAs as Regulators of Oncogene Expression

To address whether specific miRNAs might be participating in cancer through the mis-regulation of oncogenes, we scanned the 3′ untranslated regions (UTRs) of 150 well-known oncogenes for sequences with significant homology to the miRNAs identified in our microarray analysis. Potential target sites were selected based on two criteria:

(1) *Perfect complementarity between positions 2–9 of the miRNA and the oncogene*. This miRNA core sequence has been identified as critical to the activities of miRNAs and the known miRNA target sites have essentially 100% complementarity at this site (Doench *et al.* 2004).

(2) *Overall T_m of the miRNA/mRNA interaction.* In addition to the core sequence, overall binding stability between miRNAs and mRNAs has been shown to be an important indicator of miRNA activity (Doench *et al.*, 2004).

As seen in Table 8, potential target sites in the 3'UTRs of known oncogenes were identified for all of the miRNAs that were observed to be routinely differentially expressed in tumor samples. Interestingly, KRAS2, MYCL1, and CBL have multiple predicted miRNA binding sites which could provide the cooperative miRNA binding that has been implicated as an important factor in miRNA regulation (Doench *et al.* 2003); Zeng *et al.*, 2003). Many of the genes listed in Table 8 become oncogenic when they are over-expressed, thus it is conceivable that reduced expression of a miRNA could lead to up-regulation of one or more oncogenes and subsequently lead to oncogenesis.

Table 8: Cancer-related miRNAs and their putative oncogene targets

miRNA	Predicted Gene Target
let-7	RAS
let-7	C-MYC
miR-21	mutS homolog 2 (MSH2)
miR-21	v-ski sarcoma viral oncogene homolog (avian) (SKI)
miR-143	breakpoint cluster region (BCR)
miR-143	MCF.2 cell line derived transforming sequence (MCF2)
miR-143	von Hippel-Lindau tumor suppressor (VHL)
miR-143	v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog (KRAS2)
miR-143	v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog (KRAS2)
miR-143	Cas-Br-M (murine) ecotropic retroviral transforming sequence (CBL)
miR-143	Cas-Br-M (murine) ecotropic retroviral transforming sequence (CBL)
miR-145	v-myc myelocytomatosis viral related oncogene (MYCN)
miR-145	fibroblast growth factor receptor 2 (FGFR2)
miR-145	Cas-Br-M (murine) ecotropic retroviral transforming sequence (CBL)
miR-188	v-myc myelocytomatosis viral oncogene homolog 1 (MYCL1)
miR-200b	cadherin 13 (CDH13)
miR-200b	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT)
miR-219	v-myc myelocytomatosis viral oncogene homolog 1 (MYCL1)
miR-219	B-cell CLL/lymphoma 2 (BCL2)
miR-219	cadherin 1, type 1, E-cadherin (epithelial) (CDH1)
miR-331	vav 1 oncogene (VAV1)
miR-331	fibroblast growth factor receptor 1 (FGFR1)
miR-331	BCL2-antagonist/killer 1 (BAK1)
miR-331	retinoic acid receptor, alpha (RARA)
miR-331	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (SRC)

EXAMPLE 13:

Measuring the Effect of miRNAs on Oncogene Expression

Confirming miRNA target site predictions can be done in a variety of ways. In *Drosophila* and *C. elegans*, genetic approaches have been applied wherein mutations in the miRNA and the putative miRNA target site(s) are made and shown to result in similar phenotypes (Ha *et al.*, 1996; Vella *et al.*, 2004). In mammalian cells, where genetic approaches are far more difficult, reporter constructs have been used to show that the 3′ UTRs of putative target genes are regulated in cells at levels that are disproportionate to reporter vector controls that contain mutations in the putative miRNA binding sites (Lewis *et al.* 2003). In addition, vectors and oligonucleotides have been used to introduce or inhibit miRNAs in cells to determine the effects on endogenous levels of putative target genes (Lewis *et al.*, 2003; Kiriakidou *et al.* 2004). The latter approach has been undertaken to validate the miRNA target site predictions.

Synthetic miRNAs and miRNA inhibitors have been developed that can be transfected into mammalian cells to either introduce miRNAs into cells or inhibit the activity of miRNAs in cells, respectively. A synthetic miRNA and a miRNA inhibitor corresponding to let-7b were used to determine if the target site predictions were correct. In these experiments, cultured cells that express undetectable levels of the miRNA were transfected with the synthetic miRNA using siPORT™ *NeoFX*™ Transfection Agent (Ambion). Immunofluorescence assays were used to RAS and C-MYC in the transfected cells. The proteins from both oncogenes were expressed at almost three-fold lower levels in cells transfected with the synthetic miRNA than cells transfected with a Negative Control miRNA (Ambion). In a reciprocal experiment, cells that naturally express high levels of the miRNA were transfected with the let-7 miRNA inhibitor. As expected, the proteins from both oncogenes were higher in cells transfected with the miRNA inhibitor than in cells transfected with the Negative Control inhibitor (Ambion). These results are consistent with the model that the miRNA regulates the expression of the two oncogenes. These data suggest that mis-regulation of a key miRNA could participate in cancer progression by failing to regulate the expression of one or more oncogenes.

EXAMPLE 14:

miRNAs in Lupus

Systemic lupus erythematosus (SLE; Lupus) is a chronic inflammatory auto-immune disease that ultimately leads to immune complex-mediated end-organ failure. It is characterized by an over activation of CD4+ T helper cells and repression of CD8+ T cytotoxic activity, leading to an overproduction of natural antibodies and pathogenic autoantibodies. Recently several histone modifications were reported in peripheral blood mononuclear cells (PBMCs) isolated from lupus patients. Diagnosis of lupus is still frequently incorrect mainly because the symptoms vary so widely and they come and go frequently, and because the disease mimics so many other disorders. Furthermore, diagnosis does not indicate the particular therapy to be used. In the absence of a cure, present-day treatment of lupus is still primarily tailored to symptomatic relief and not to the diagnosis. A diagnostic assay with high specificity and sensitivity would be very important.

Samples were analyzed from 16 individuals, 8 with clinically verified lupus and 8 non-lupus patients that were age- and gender-matched with the lupus patients. Total RNA from these samples was isolated using the glass fiber filter method described above. The total RNA was fractionated by tube electrophoresis to recover the miRNAs. The miRNAs were fluorescently labeled with Cy3 or Cy5 using the two-step fluorescent labeling process described above. The labeled miRNAs were hybridized to miRNA probes arrayed on glass slides as described above. The signal from the hybridized miRNAs was quantified using a GenePix 4000B Scanner (Axon) and the lupus and normal sample signals were compared to identify differentially expressed miRNAs. Each array experiment included duplicate arrays.

Fourteen miRNAs were differentially expressed in all of the lupus samples relative to the matched samples. miR-301, miR-199, miR-95, miR-105, mu-miR-290, miR-215, miR-188, miR-186, miR-211,miR-331, and miR-137 were expressed at 50% or less in the lupus samples than the corresponding normal samples. miR-21, miR-223, and miR-342 were expressed at 50% or greater in the lupus samples than the corresponding normal samples. Several of the miRNAs were differentially expressed by as much as ten-fold between the lupus and normal samples. These miRNAs represent targets for diagnostic assay of therapeutic development.

EXAMPLE 15:

miRNAs and Prion Diseases

Novel infectious particles, termed prions, composed largely and perhaps solely of a single protein, are the likely causative agents of a group of transmissible spongiform encephalopathies that produce lethal decline of cognitive and motor function. Evidence indicates that the responsible protein arrives at a pathogenic state by misfolding from a normal form that has ubiquitous tissue distribution.

Using two cell-based prion model systems, the identification of miRNAs that might be associated with the process was pursued. One model system comprises two cell lines, one of which is susceptible to prion formation and one that is not. The second model system involves cells before and after they have been infected with prions. Total RNA from prion-sensitive cells, prion-insensitive cells, and prion-infected cells was isolated using the glass fiber filter method described above. The total RNA was fractionated by tube electrophoresis to recover the miRNAs. The miRNAs were fluorescently labeled with Cy3 or Cy5 using the two-step fluorescent labeling process described above. The labeled miRNAs were hybridized to miRNA probes arrayed on glass slides as described above. The signal from the hybridized miRNAs was quantified using a GenePix 4000B Scanner (Axon) and the signal from each of the samples was compared to identify differentially expressed miRNAs.

As seen in FIG. 13, ten miRNAs were significantly up- or down-regulated in both prion-sensitive and prion-infected cells relative to prion resistant, uninfected cells. Arrays on multiple biological replicates for both model systems have confirmed these results. Based on their expression profiles, miR-95, 135a, 7, 9, 27a, 130a, 16, 26a, and 24 likely are involved directly or indirectly in prion infection and might represent diagnostic or therapeutic targets for prion disease.

EXAMPLE 16:

Stroke-Associated miRNAs

Stroke is a major cause of death and permanent disability in humans. They occur when blood flow to a region of the brain is obstructed and may result in death of brain tissue. There are two main types of stroke: ischemic and hemorrhagic. Ischemic stroke is caused by blockage in an artery that supplies blood to the brain, resulting in a deficiency in blood flow (ischemia). Hemorrhagic stroke is caused by the bleeding of ruptured blood vessels (hemorrhage) in the brain. Understanding miRNAs involved in stroke might enhance detection and/or treatment.

A stroke model system was used wherein mice are "preconditioned" by reducing oxygen flow to the brain (Kitagawa 1991). An equivalent set of six mice were used; three were preconditioned and three were untreated. 24 hours after pre-conditioning, the mice were sacrificed. Total RNA from these samples was isolated using the glass fiber filter method described above. The total RNA was fractionated by tube electrophoresis to recover the miRNAs. The miRNAs were fluorescently labeled with Cy3 or Cy5 using the two-step fluorescent labeling process described above. The labeled miRNAs were hybridized to miRNA probes arrayed on glass slides as described above. The signal from the hybridized miRNAs was quantified using a GenePix 4000B Scanner™ (Axon) and the preconditioned and normal sample signals were compared to identify differentially expressed miRNAs.

Analysis of the miRNA profiles of the preconditioned animals (labeled P1, P2, and P4) revealed 10 miRNAs that were expressed at significantly different levels in all three pre-conditioned animals relative to the three non-treated animals (FIG. 14). These miRNAs resulted from ischemic pre-conditioning and represent potential targets for stroke diagnosis, prevention, or treatment.

EXAMPLE 16:

Synthetic miRNA Library Screens for miRNAs That Influence Cell Proliferation and Cell Viability in Various Cell Types

A hallmark of cancer is uncontrolled cell proliferation; cell proliferation assays are commonly used by researchers to study the influence of genes in oncogenesis. A cell proliferation assay was used in conjunction with the miRNA inhibitor library to identify miRNAs that influence cell proliferation.

HeLa (human ovarian cancer) and A549 (human lung cancer) cells were transfected in triplicate with 150 synthetic miRNAs using siPORT NeoFX (Ambion) according to the manufacturer's instructions. The 150 are as follows: Let-7a, Let-7b, Let-7c, Let-7d, Let-7g, mir-1, mir-7, mir-9, mir-10a, mir-10b, mir-15a, mir-16, mir-18, mir-19a, mir-17-3p, mir-20, mir-21, mir-22, mir-23a, mir-23b, mir-24, mir-25, mir-26a, mir-27a, mir-28, mir-29a, mir-31, mir-32, mir-30a-3p, mir-34a, mir-92, mir-95, mir-96, mir-98, mir-99a, mir-100, mir-101, mir-103, mir-105, mir-107, mir-108, mir-122, mir-124, mir-125a, mir-125b, mir-126, mir-128, mir-129, mir-132, mir-133A, mir-133B, mir-134, mir-135, mir-136, mir-137, mir-139, mir-140, mir-141, mir-142, mir-143, mir-144, mir-145, mir-146, mir-147, mir-148, mir-149, mir-150, mir-151, mir-152, mir-153, mir-155, mir-181a, mir-182, mir-183, mir-184, mir-186, mir-187, mir-188, mir-190, mir-191, mir-192, mir-193, mir-194, mir-195, mir-196, mir-197, mir-198, mir-199, mir-201, mir-203, mir-204, mir-205, mir-206, mir-207, mir-208, mir-210, mir-211, mir-212, mir-214, mir-215, mir-216, mir-217, mir-218, mir-219, mir-220, mir-221, mir-223, mir-224, mir-299, mir-301, mir-302, mir-320, mir-322, mir-323, mir-325, mir-324-3p, mir-328, mir-330, mir-331, mir-335, mir-337, mir-338, mir-339, mir-340, mir-345, mir-346, mir-367, mir-368, mir-369, mir-370, mir-371, mir-372, mir-373, mir-374, mu-mir-290, mu-mir-291, mu-mir-292-3p, mu-mir-293, mu-mir-294, mu-mir-295, mu-mir-297, mu-mir-298, mu-mir-329, mu-mir-341, mu-mir-344, mu-mir-351, mu-mir-376b, mu-mir-380-3p, mu-mir-409, mu-mir-411, mu-mir-412

The synthetic miRNAs were double stranded nucleic acid molecules composed of an active strand and a complementary strand. The active strand contained a sequence that was identical to the corresponding mature miRNA. The complementary strand contained a sequence that was 100% complementary to the relevant region of the mature miRNA sequence, but 1) lacking two nucleotides on its 3' end that were complementary to the mature miRNA sequence (at the 5' end of the active strand) and 2) having a dinucleotide overhang on its 5' end with respect to the active strand. In other words, the two strands were fully complementary to the other's sequence except that each strand has a dinucleotide 5' overhang with respect to the other strand. The same kind of synthetic miRNAs were used for Examples 17-20 as well. Any exceptions are described below.The miRNAs indicated in the tables identify the miRNA that corresponds to the provided synthetic sequence.

Jurkat cells (human leukemia cell) and primary human T-cells in triplicate were electroporated with the same set of synthetic miRNAs using siPorter-96 (Ambion) according to the manufacturer's instruction. All cells were analyzed for viable and non-viable cells 72 hours post-transfection using the PCA-96 (Guava) with the Viacount Assay. Viable cell number is the number of live cells in a well at the point of the assay. The numbers provided in the tables below are equal to the average number of viable cells in wells transfected with a particular miRNA divided by the number of viable cells in wells transfected with negative control synthetic miRNAs multiplied by 100 to yield the % Cell Viability of miRNA-transfected cells relative to negative control transfected cells.

Significance was assigned based on the average values of the negative control transfecetd samples. miRNAs that were significantly different than the negative controls were qualified as "significant" based on being at least two standard deviations above or below the negative control data.

The sequence if miRNA-325 is 5'- ccuaguagguguccaguaagugu-3'.

TABLE 9

miRNAs That Significantly Reduce Cell Viability of HeLa Cells

	% Viability	std dev
mir-345	75	5.9
mir-346	77.8	8.2
mir-193	79.6	14.7

mir-206	79.6	6.5
mir-337	80.8	3.1
mmu-mir-293	82.6	1.7
mir-299	84.0	4.0
mmu-mir-329	84.5	4.5
mmu-mir-409	86	2.8
mmu-mir-292-3p	86.2	2.8
mir-210	86.4	5.1
mmu-mir-344	86.4	5.3
mmu-mir-298	86.7	4.2
mir-208	87.4	4.5
mir-197	87.6	7.5
mir-217	87.9	3.5
mir-1	88.2	9.0
mir-124	88.8	4.2

TABLE 10

miRNAs That Significantly Reduce Viable Cell Number of HeLa Cells

	Total Cell	std dev
Let-7b	16.2	8.1
Let-7g	22.7	8.2
Let-7c	24.1	7.2
mir-124	24.5	3.4
Let-7a	25.4	1.2
Let-7d	37.3	2.3
mir-337	37.5	16.9
mir-1	38.7	2.2
miR-299	38.9	4.2
mir-34a	40.5	13.3
mmu-mir-292	41.2	8.3
mir-122	41.2	6.5
mir-346	41.9	4.3
mir-101	43.4	6.4
mir-210	47.1	8.4
mir-147	47.7	8.2
mir-98	50.6	2.6
mir-345	51.8	6.8
miR-92	52.4	6.8
miR-96	53.2	0.9
mir-7	54.0	5.3
mir-133b	55.9	3.1
mir-206	56.0	12.4
mmu-mir-297	56.0	5.7
mir-19a	57.2	20.6
mmu-mir-344	57.5	14.1
mir-205	58.9	18.7
mir-208	60.5	11.1

TABLE 11

miRNAs That Significantly Increase Viable Cell Number of HeLa Cells

	Total Cell	Std dev
mir-32	142.9	25.4
mu-miR-290	143.5	17.6
mir-212	143.5	10.4
mir-92	144.7	16.8
mir-323	147.3	25.9
mir-145	148.1	22.2
mir-324	148.2	9.0
mir-198	152.1	67.8
mir-27a	156.2	13.4
mir-369	158.4	27.3
mir-31	159.3	16.1
mir-335	161.7	20.8
mmu-mir-351	162.3	6.9
mir-370	164.3	4.5
mir-325	169.6	19.8
mir-331	172.5	24.0
mir-139	181.3	11.2

TABLE 12

miRNAs That Significantly Reduce Cell Viability of A549 Cells

	% Viability	St dev
mir-193	92.4	2.5
mir-224	92.5	1.4
mir-96	92.6	0.1
mir-346	93.9	1.6
mmu-mir-293	94.9	0.7
mir-34a	95	0.2
mir-216	95.1	1.0
mmu-mir-380	95.2	0.8
mir-182	95.6	0.8
mir-301	95.6	1.0
mmu-mir-344	95.8	0.2
mmu-mir-409	95.8	0.6
mir-369	95.9	0.7

TABLE 13

miRNAs That Significantly Reduce Viable Cell Number in A549 Cells

	Cell Number	St Dev
mir-124	44.3	2.2
mir-16	52.9	1.3
mir-337	54.7	7.0
mir-195	59.3	6.7
mir-34a	60.8	2.1
mir-15a	60.9	3.7
mir-28	61.3	0.8
Let-7g	61.9	0.8
mmu-mir-292	62.2	2.3
mmu-mir-344	62.6	9.1
mir-7	62.9	4.6

mir-193	63.7	3.3
mir-137	63.9	1.3
mir-147	64.8	0.5
mir-29a	67.0	3.8
mir-129	67.2	3.3
mir-22	67.5	3.4
mir-126	68.0	2.6
mir-345	69.2	7.4
mir-192	69.5	5.9
Let-7b	70.2	2.2
Let-7d	70.5	2.7
mir-346	70.9	7.1

TABLE 14

miRNAs That Significantly Increase Viable Cell Number in A549

	Total cell	Std dev
mir-373	110.4	7.9
mir-25	111.8	6.0
mmu-mir-294	112.1	5.9
mir-32	120.8	4.3
mir-92	122.4	4.0

TABLE 15

miRNAs That Significantly Reduce Cell Viability of Jurkats Cells

	% Viability	St Dev
let-7a	20.54	0.70
miR-10b	35.98	2.92
let-7b	48.79	5.08
miR-17-3p	61.55	15.63
miR-30a-3p	64.36	26.60
miR-34a	65.45	20.44
miR-122	65.63	17.80
miR-29a	66.44	7.14
miR-101	67.44	29.56
miR-133a	71.51	17.82
miR-19a	71.77	23.79
miR-32	75.59	11.69
miR-1	75.74	12.92
miR-132	76.32	16.22
miR-28	77.07	16.58
miR-20	77.60	15.23
miR-134	78.96	1.75

TABLE 16

miRNAs That Significantly Increase Cell Viability in Jurkat Cells

miR-181-a	Total cell	Std dev

	122.77	22.40
miR-9	124.63	9.98
miR-141	126.08	24.03
miR-98	126.24	11.90
miR-10a	126.86	8.93
miR-125b	128.71	3.50
miR-126	130.69	18.20
miR-100	130.77	14.60
miR-23b	132.18	3.50
miR-140	135.73	4.08
miR-155	142.57	22.40
miR-15a	143.01	11.29
miR-129	146.94	9.92
miR-25	150.25	17.85
miR-143	158.74	1.86
miR-26a	166.09	13.65

TABLE 17

miRNAs that Significantly Reduce Cell Viability in Primary T-Cells

	% Viability	St Dev
miR-184	61.04	12.16
miR-145	68.98	11.23
miR-186	69.64	6.99
miR-139	69.85	0.29
miR-134	71.90	22.42
miR-190	75.59	2.43
miR-144	77.13	4.18
miR-183	77.71	2.86
miR-147	78.09	0.33
miR-140	78.70	5.81
miR-155	79.26	10.68

TABLE 18

miRNAs that Significantly Increase Cell Viability of Primary T-Cells

	% Viability	St Dev
miR-126	120.81	40.08
miR-10b	121.28	18.86
miR-17	122.46	3.71
miR-10a	124.11	9.46
miR-20	124.75	13.60
let-7c	124.81	4.00
miR-125a	125.66	5.13
miR-15a	129.07	10.96
let-7b	130.11	13.48
let-7a	130.88	16.16
miR-18	131.73	1.75

It is interesting to note that the miRNAs that affect one cell type often fail to affect other cell types. This is likely due to the fact that the cellular processes that are active vary between different cell types. This can be vitally important when considering the potential of miRNA-based therapeutics. Abnormal (disease) cells are different from normal cells owing to the fact that different cellular processes are active in the two cell types. Identifying miRNAs that have differential effects on normal and abnormal cells would be ideal since they could be delivered globally and expected to have an effect on only disease cells. When the cell viability data were compared for the leukemia (cancerous T-cell) cells and primary T-cells, it was noted that let-7a, let-7b, and miR-10b all significantly reduce the percentage of viable cells in the leukemia cells while essentially having no effect on the corresponding normal T-cells. These miRNAs are candidates for leukemia drugs.

EXAMPLE 17

Synthetic miRNA library screen for miRNAs that influence ERK activation

In order for cancer cells to proliferate they must subvert both the machinery that controls the cell division cycle and the process of programmed cell death (apoptosis). This is frequently achieved by mutation of specific proto-oncogenes such as *Ras* or tumor suppressors such as *p53*. The Ras-family of membrane associated GTPases transmit signals into the interior of the cell by the activation of a number of cytosolic signal transduction pathways such as the Raf>MEK>ERK MAP kinase signaling pathway. Disregulation of the Ras/Raf/MEK/ERK pathway plays a major role in cancer pathogenesis (Meijer).

To identify miRNAs that affect ERK activation, HeLa cells were transfected in a 96-well plate format with 150 different synthetic miRNAs. Prior to transfection, the HeLa cells were trypsinized to remove adherent cells and diluted in normal growth medium to 10^5 cells/mL. 0.5 μl of siPort NeoFX in 9.5 μl of Optimem I medium was added to the cells and incubated for 10 minutes at room temp (10 μL for each sample). miRNAs were rehydrated with 10 μl of diluted siPORT NeoFX. The samples were incubated at 37 °C and then the transfected samples were evaluated 72 hours after transfection.

The controls for ERK activation were performed by depriving the wells of a phosphate source for detection of ERK phosphorylation. 100 μl of serum-free media (DMEM) to 37°C was added per well and the cells were incubated for 4 hours at 37 °C to attain basal phosphorylation levels. For the positive control wells, serum-free media was aspirated from wells and 100 µL of 100 ng/mL EGF was added before incubating the cells for 7.5 minutes at 37 °C.

Media from all wells was removed by aspiration and the cells were immediately fixed in 150 µL of 3.7% Formaldehyde in 1X PBS for 20 minutes at room temp with no shaking. Fixing solution was removed to an appropriate waste container. The fixed cells were washed three times with 1X PBS. The wells were then washed three times with 200 µL of 1X PBS containing 0.1% Triton X-100 for 5 minutes per wash, with shaking at room temp.

Cells were blocked by adding 150 µL of Li-COR Odyssey Blocking Buffer to each well. The solution was moved carefully by pipetting down the sides of the wells to avoid detaching the cells. Blocking was for 90 minutes at room temp with moderate shaking on a rotator and the two primary antibodies were added to a tube containing Odyssey Blocking Buffer. The primary antibody was incubated for 2 hours with gentle shaking at room temp (Phosho-ERK (Rabbit, 1:100 dilution; Cell Signaling Technology 9101). Total ERK2 (Mouse; 1:75 dilution; Santa Cruz Biotechnology SC-1647)). The wells were washed three times with 1X PBS + 0.1% Tween™-20 for 5 minutes at room temp with gentle shaking, using a generous amount of buffer. The fluorescently labeled secondary antibody was diluted in Odyssey Blocking Buffer (Goat anti-rabbit Alexa Fluor 680 (1:200 dilution; Molecular Probes) Goat anti-mouse IRDye 800CW (1:800 diution; Rockland Immunochemicals)). The antibody solutions were mixed well and 50 µL of the secondary antibody solution was added to each well. The antibody solution was incubated for 60 minutes with gentle shaking at room temp. The plate was washed three times with 1X PBS + 0.1% Tween-20 for 5 minutes at room temp with gentle shaking, using a generous amount of buffer. After a final wash, wash solution was completely removed from wells. The plates were scanned with the Odyssey Infrared Imaging System (700 nm detection for Alexa Fluor 680 antibody and 800 nm detection for IRDye 800CW antibody). Negative control transfected cells yield 100% erk activation (meaning background levels of active erk). Transfecting cells with some of our miRNAs alters the level of active erk.

TABLE 19

miRNAs That Activate ERK

miR	% Activation	Std Dev
mir-218	312.96	22.91
mir-210	291.74	38.23
mir-217	273.49	26.84
mir-152	265.54	35.82
mir-148	264.38	43.55
mir-223	264.15	39.72
mir-301	261.36	61.77
mir-328	259.48	45.87
mir-206	255.51	55.53
mir-125a	252.46	27.34
mmu-mir-329	243.38	5.43
mir-19a	241.52	31.33
mir-25	238.90	44.94
mmu-mir-294	235.51	24.60
mir-212	231.36	23.61
mmu-mir-295	221.47	14.05
mir-370	220.60	22.88
mir-216	219.17	25.98
mir-96	213.93	57.07
mir-339	213.9	42.25
mir-134	211.15	12.84
mir-372	211.13	5.67
Positive Control	245.36	10.76

EXAMPLE 18

Screen for miRNAs that influence apoptosis

Apoptosis is a natural cellular process that helps control cancer by inducing death in cells with oncogenic potential. Many oncogenes function by altering induction of apoptosis. To identify miRNAs that participate in apoptosis, an apoptosis assay was used with the miRNA inhibitor library.

HeLa cells (8000 cells/well of 96 well plate) were transfected in triplicate with more than 150 synthetic miRNAs (described above) (3 pmoles) using Ambion siPORT™ *NeoFX*™. The media was changed 24 hrs after transfection and cells were processed 72 hrs after transfection. The cells were measured for apoptosis by measuring caspase 3 activity as follows: 1) Cells were washed once with PBS and frozen at –80°C. 2) Cells were lysed by adding 40 µl of cold lysis buffer (50 mM HEPES pH 7.2, 40 mM NaCl , 0.5% NP40, 0.5 mM EDTA) to the wells and incubated for 20 min at 4°C. 3) Add 160 ul ICE buffer (50 mM HEPES pH 7.4, 0.1%

CHAPS, 0.1 mM EDTA, 10% sucrose) + 5 mM DTT containing 20 uM DEVDafc substrate. 4) Measure fluorescence increase in one hour at 400 ex, 505 em.

Samples were also analyzed for cell number using a general esterase assay to normalize the caspase 3 results. FDA substrate (0.4 mg/ml fluorescein diacetate (FDA) in acetonitrile) was diluted 1:19 into dilution buffer (40 mM TrisCl pH 7.5, 20 mM NaCl, 0.5% NP-40, 0.02 mg/ml final conc). 40 µl buffer (40 mM TrisCl pH 7.5, 0.5% NP-40) was added to each sample well. Samples were incubated 10 min on ice. 160 ul of diluted FDA substrate was added to each well. Fluorescence was measured for 30 min at 37 deg (ex = 488, em = 529). The slope of fluorescence increase over time is a function of the cell number in the plate.

miRNAs that affect apoptosis are listed in the table below. These miRNAs apparently regulate pathways that lead to apoptosis. Mis-regulation of these miRNAs could induce cells to undergo apoptosis or might keep the cells from undergoing apoptosis. Introducing or inhibiting these miRNAs in cancer (or other disease) cells that have overcome apoptotic signaling pathways or Parkinson's (or other disease) cells that have prematurely induced apoptosis could be used to treat the diseases.

TABLE 20

miRNAs that Significantly Increase the Percentage of Apoptotic Cells

	Relative change in apoptotic cells	St Dev
mir-338	773.46	69.82
mir-27a	607.24	150.08
mir-128	594.42	260.06
mir-23a	473.44	208.82
mir-324	442.99	101.03
mir-22	439.13	62.59
mir-181a	409.97	65.14
mmu-mir-293	403.86	53.41
mmu-mir-412	402.27	42.04
mir-196	378.13	28.15
mir-31	373.90	61.39
Let-7d	369.10	88.94
mir-23b	360.68	81.97
mu-miR-290	354.90	46.63
mir-217	347.38	56.49
mir-199	345.75	67.55
mir-24	317.43	62.85
mir-214	312.25	7.38
mir-198	303.24	44.25

TABLE 21

miRNAs that Significantly Decrease the Percentage of Apoptotic Cells

	Relative change in apoptotic cells	St Dev
mir-105	39.97	8.91
mir-34a	37.75	8.41
mir-96	31.89	13.40
mmu-mir-292	30.72	4.27
mir-126	28.71	4.24
mir-137	12.69	11.80
mir-101	7.50	6.91

EXAMPLE 19

Synthetic miRNA library screen for miRNAs that influence hTert expression

Telomerase is a complex of proteins and RNA that maintains the ends of chromosomes by appending telomeres. With rare exceptions, terminally differentiated cells lack active telomerase. One of the exceptions is cancer cells. More than 90% of human cancer samples have active telomerase (reviewed in Dong *et al.* 2005). The hTert gene encodes the catalytic domain of telomerase. The expression of hTert correlates with telomerase activity in cells making it a good surrogate for telomerase activity. An RT-PCR based assay for monitoring hTert mRNA expression in telomerase negative cells has been developed and used to identify miRNAs that participate in the regulation of telomerase. The miRNAs that regulate telomerase activity represent intervention points for cancer therapeutics.

BJ cells are normal human foreskin fibroblasts that lack hTert mRNA and telomerase activity. BJ cells were trypsinized and diluted to 13,000 cells/ml in normal growth media. 0.3 μl of lipofectamine 2000 agent was diluted into 40 μl of OPTI-MEM and incubated for five minutes. The diluted transfection reagent was added to the wells of 96-well plates that contained 150 synthetic miRNAs (as described above) as well as two different negative control synthetic miRNAs. Each well housed a different synthetic miRNA. The synthetic miRNAs and transfection agent were incubated for 15 minutes at room temperature and then 200 μl (2,600 cells) were added on top of the lipid/miRNA complex. Cells were placed in an incubator and RNA was isolated 72 hours later. RNA was isolated from the cells in each well using RNAqueous™-MagMAX96 Total RNA Isolation kit (Cat#1830) standard protocol (lyse cells in wells). Reverse transcription was done using the

RETROscript^{TM} reaction by adding 11 μl of total RNA (20-100 ng/μl) to 1 ul of random decamers and incubated in 70°C water bath for 3 minutes then place on ice. Next, 8ul of the cocktail containing Nuc-free water 3.8 μl, 10X Reverse Transcription buffer 2.0ul, 2.5mM dNTPs 2.0ul, RNase Inhibitor Protein (40U/ul), 0.1ul MMLV-RT (100 U/μl), and incubated at 42°C for 1 hour, then 92°C for 10 minutes.

Real time PCR reactions were assembled to quantify hTert mRNA and 18S rRNA in each of the samples. Nuclease-free water, 10X Complete PCR buffer/SYBR, 25mM MgCl2, 2.5mM dNTPs, 50X ROX, 18S- or hTert-specific primers (for & rev mix 3uM), cDNA from the various samples, and Super taq polymerase into a PCR tube. The reaction was heated to 95°C for 5 minutes and then subjected to 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, 72°C for 30 seconds. The amplification products were monitored using the ABI 7600^{TM} (Applied Biosystems). BJ cells ordinarily fail to yield amplification products with the hTert primers. Those miRNA-transfected samples that yielded a hTert PCR product were also analyzed for 18S rRNA lavels to ensure that there were not significantlyt more cells in the samples that might have contributed to the amount of hTert in the samples.

The hTert mRNA was detected in duplicate transfections of each of the miRNAs listed below. These miRNAs presumably affect pathways that regulate the expression of the hTert gene. Over-expression of any of these miRNAs might contribute to cancer by activating telomerase. Regulating the activities of these miRNAs in cancer cells could limit their transformation and overcome oncogenesis.

TABLE 22

hTert Activators
mmu-mir-295
mir-92
mir-337
mir-26a
mir-224
mir-21
mir-195
mir-16
mir-15a
mir-128
mir-125b
mir-125a
mir-105

EXAMPLE 20

Synthetic miRNA library screens for miRNAs that influence cell cycle

The adult human body consists of about 50-100 trillion cells. Each day, several billion of these cells divide in two to replace the billions of cells that die and are removed. In the course of an average lifetime, this adds up to an astronomical number of cell divisions, most of which go perfectly well. Errors do occur, however, and if they are not corrected they may lead to cancer. Cell growth and division are normally controlled by an intricate system of checks and balances. But occasionally a cell will start to proliferate wildly, dividing again and again and defying all normal restraints on its growth. That is the beginning of most common forms of cancer.

The inventors transfected 4,000 BJ cells/well in triplicate with 46 synthetic miRNAs using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The

let7a
let7a
mir1
mir1
mir105
mir125a
mir128
mir142
mir145
mir146
mir147
mir150
mir15a
mir16
mir186
mir187
mir188
mir191
mir195
mir20
mir206
mir21
mir211
mir223
mir224
mir26a
mir320
mir324-3p
mir325
mir335
mir337

mir338
mir345
mir371
mir373
mir92
mmu201
mmu207
mmu290
mmu291-3p
mmu294
mmu295
mmu297
mmu322
mmu376b
mmu409

24 hours post-transfection, half of the BJ cells from each well were removed to fresh medium. 72 hrs post-transfection, the cells were fixed with 4%paraformaldehyde at a final concentration of 2%. The fixed cells were stained with propidium iodide (TTP LabTech protocol) and assessed using the TTP LabTech cell scanner. Propidium iodide stains DNA and the relative DNA content in a cell corresponds with its position in the cell cycle. The cell scanner measured propidium iodide staining in each cell and assigned its position in the cell cycle. The percentage of cells in each stage of cell cycle was calculated and compared to cells transfected with negative control synthetic miRNAs. The relative change in cells in each stage was calculated for each miRNA that was used. Those synthetic miRNAs that induced a significant shift toward or away from a specific stage of cell cycle are listed below. These represent miRNAs that regulate key points in the cell cycle and offer key intervention points for cancer-related therapeutic development.

TABLE 23

miRNAs that significantly reduce the percentage of BJ cells in G1 phase of the cell cycle

miRNA	% Diff in Cells in G1	St. Dev.
mir-21	54.4	4.2
mir-20	63.6	9.3
mir-1	65.3	9.5
mir-206	66.8	9.0
mir-373	72.6	5.7
mir-26a	78.0	4.0

TABLE 24

miRNAs that significantly increase the percentage of BJ cells in G1 phase of the cell cycle

miRNA	% Diff in Cells in G1	St. Dev.
rno-mir-325	121.7	5.3
mmu-409	123.2	13.7
mir-324	123.7	4.9
mir-195	125.1	2.5
mmu-376b	126.5	3.1
mir-142	127.0	13.0
mir-371	128.9	2.8
let-7a	131.5	4.5
mir-146	141.5	7.7
mir-128	143.0	2.4

TABLE 25

miRNAs that significantly reduce the percentage of BJ cells in S phase of the cell cycle

miRNA	% Diff in Cells in S	St. Dev.
mir-128	55.5	3.8
let-7a	57.6	8.7
mir-142	59.5	24.7
mir-146	63.5	16.8
mmu-297	65.0	14.1
mir-337	65.3	11.3
mir-195	65.6	0.1
mmu-376b	69.1	11.6
mir-324	72.2	9.4
mir-187	72.3	10.9
mir-186	72.8	6.1

TABLE 26

miRNAs that significantly increase the percentage of BJ cells in S phase of the cell cycle

miRNA	% Diff in Cells in S	St. Dev.
mir-92	132.0	14.7
mir-15a	134.8	13.9
mir-191	135.9	29.1
mir-26a	136.0	7.6
mir-20	139.7	17.6
mmu-290	141.0	11.7
let-7a	141.1	19.9
mir-345	143.3	45.8
mir-16	150.1	24.8
mir-224	150.6	9.8

TABLE 26

miRNAs that significantly reduce the percentage of BJ cells in G2/M phase of the cell cycle

miRNA	% Diff in Cells in G2/M	St. Dev.
mir-147	51.2	6.1
mir-371	52.8	2.7
mir-146	57.2	5.3
mir-195	58.9	4.4
mir-128	65.4	2.7
mir-15a	67.4	13.7
let-7a	69.1	2.8

TABLE 27

miRNAs that significantly increase the percentage of BJ cells in G2/M phase of the cell cycle

miRNA	% Diff in Cells in G2/M	St. Dev.
mir-26a	130.2	5.8
mir-187	132.0	4.3
mir-145	136.8	13.7
mir-373	137.9	5.2
mir-20	143.0	10.6
mir-21	160.3	7.1

TABLE 28

miRNAs that significantly increase the percentage of BJ cells with greater than 2X amount of DNA

miRNA	% Diff in Cells w/>2X DNA	St. Dev.
mir-20	157.9	23.4
mir-1	161.9	13.6
mir-345	176.1	17.4
mir-373	177.9	32.7
mir-337	195.0	52.1
mir-21	209.4	45.7

EXAMPLE 21

Synthetic miRNA library screen for miRNAs that influence cell proliferation

Cell proliferation assays were used in conjunction with our synthetic miRNA library to identify miRNAs that influence cell proliferation in a broad range of cells, including those from lung, breast, prostate, skin, cervix, T-cell, and foreskin tissues.

Cervical (HeLa), lung (A549, CRL-5826, and HTB-57), breast (MCF12A and BT549), prostate (22Rv1), T-cells (Jurkat and primary normal), and skin (TE354T, TE353SK, and BJ) cells were transfected in triplicate with each of the more than 150 synthetic miRNAs in our library. With the exceptions of Jurkats and Primary T-cells, each cell type was transfected with 5 picomoles of each of the miRNAs in the synthetic miRNA library using siPORT™ *NeoFX*™ (Ambion) at a plating density of approximately 8000 cells/well of 96 well plate. The Jurkats and primary T-cells were mixed at a rate of approximately 50,000 cells/well with 500 picomoles of each of the synthetic miRNAs. The media was changed 24 hrs after transfection. 72 hours post-transfection, cell number was estimated by one of three methods:

(1) Alamar blue was added to each well and the 96-well plates were analyzed using a plate reader. Alamar blue is a substrate for a metabolic enzyme in cells and the reaction product is fluorescent. The fluorescence in each well correlates with the total number of cells in each well.

(2) ViaCount Flex Reagent™ (Guava), a dye that fluoresces when it interacts with DNA, was added to each well and fluorescence was quantified using the Guava PCA-96™ according to the manufacturer's instructions.

(3) Propidium iodide, a dye that fluoresces when it interacts with DNA, was added to each well and the total number of cells in the well was estimated by counting unique sites of stained DNA using the TTP LabTech Cell Scanner according to the manufacturer's instructions.

The impact of each miRNA on cell proliferation was assessed by dividing the cell number reading of each well by the average cell number reading for wells transfected with a negative control (NC) miRNA.

Presented in FIG. 15A-C are synthetic miRNAs that significantly reduced the proliferation of the various cell types that were analyzed. These miRNAs represent molecules that could be used for therapeutics, diagnostics, creating cell lines with interesting research properties, and inducing differentiation.

Approximately 10% of the miRNAs significantly reduced cell proliferation for at least four different cell types. These miRNAs (presented in ranked order in the table below) are provided below and can be implemented in methods and compositions of the invention.

Table 29

Common Anti-Proliferation miRNAs

miRNA	# Positives
miR-124	7
miR-16	6
miR-101	6
miR-126	6
miR-147	6
miR-15a	5
miR-96	5
miR-105	5
miR-142	5
miR-215	5
miR-346	4
miR-206	4
miR-192	4
miR-194	4

Among the cells that were used in the synthetic miRNA library screens are matched pairs of cancer and non-cancer cells from breast, skin, and T-cell. Interestingly, many synthetic miRNAs differentially affected proliferation in the cell pairs (see table below).

Table 30

Breast

	Cancer		**Non-Cancer**	
miRNA	%NC	% Std Dev	%NC	% Std Dev
mir-201	79	14	103	17
mir-192	81	3	95	17
mir-92	85	11	104	24

Skin

	Cancer		**Normal**	
pre-MIR	% of NC	%ST DEV	% of NC	%ST DEV
mir-154	51	5	93	10
mir-195	58	3	87	5
mu-mir-376b	65	3	99	8
mir-201	67	8	106	4
mir-26a	69	12	97	17
mir-193	69	4	105	10

T-Cell

	Leukemia		**Normal**	
	%NC	% St Dev	%NC	% St Dev
let-7a	21	1	137	15
let-7b	50	5	136	13
miR-101	69	30	95	5
miR-10b	37	3	115	18
miR-122	67	18	104	18
miR-17-3p	63	16	116	4
miR-29a	68	7	111	8
miR-30a-3p	66	27	97	18
miR-34a	67	21	100	1

Presented in FIG.16are synthetic miRNAs that significantly increase the proliferation of the various cell types that were analyzed.

EXAMPLE 22

miRNA inhibitor library screens identify miRNAs that influence cell proliferation

A cell proliferation assay was used in conjunction with our synthetic miRNA library to identify miRNAs that influence cell proliferation in a broad range of cells, including those from lung, breast, prostate, skin, cervix, T-cell, and foreskin tissues.

Breast (MCF12A), prostate (22Rv1), lung (A549), and skin (TE354T) cells were transfected in triplicate with each of the more than 150 miRNA inhibitors in our library. Each cell type was transfected with 10 picomoles of each of the miRNA inhibitors in the library using siPORT™ *NeoFX*™ (Ambion) at a plating density of approximately 8000 cells/well of 96 well plate. 72 hours post-transfection, cell number was estimated by one of three methods:

(1) Alamar blue was added to each well and the 96-well plates were analyzed using a plate reader. Alamar blue is a substrate for a metabolic enzyme in cells and the reaction product is fluorescent. The fluorescence in each well correlates with the total number of cells in each well.

(2) ViaCount Flex Reagent (Guava), a dye that fluoresces when it interacts with DNA, was added to each well and fluorescence was quantified using the Guava PCA-96 according to the manufacturer's instructions.

(3) Propidium iodide, a dye that fluoresces when it interacts with DNA, was added to each well and the total number of cells in the well was estimated by counting unique sites of stained DNA using the TTP LabTech Cell Scanner according to the manufacturer's instructions.

The impact of each miRNA inhibitor on cell proliferation was assessed by dividing the cell number reading of each well by the average cell number reading for wells transfected with a negative control (NC) miRNA.

Presented in FIG. 17 are miRNAs whose inhibition significantly reduced the proliferation of the various cell types that were analyzed. These miRNAs represent molecules that could be used for therapeutics, diagnostics, creating cell lines with interesting research properties, and inducing differentiation.

Presented in FIG. 18 are miRNA inhibitors that significantly increase the proliferation of the various cell types that were analyzed. These miRNAs represent molecules that could be used for therapeutics, diagnostics, creating cell lines with interesting research properties, and inducing differentiation.

EXAMPLE 23

Synthetic miRNA library screen for miRNAs that influence cell viability

The basis for most human diseases is the subversion of one or more cells to function in ways that are outside what they normally do. For instance, cancer initiates with the immortalization and transformation of a single cell which then divides repeatedly to form a tumor. Compounds that reduce the viability of disease cells are used routinely to treat patients with cancer and other diseases.

Cervical (HeLa), lung (A549), and T-cells (Jurkat and primary normal) were transfected in triplicate with each of the more than 150 synthetic miRNAs in our library. With the exceptions of Jurkats and Primary T-cells, each cell type was transfected with 5 picomoles of each of the miRNAs in the synthetic miRNA library using siPORT™ *NeoFX*™ (Ambion) at a plating density of approximately 8000 cells/well of 96 well plate. The Jurkats and primary T-cells were mixed at a rate of approximately 50,000 cells/well with 500 picomoles of each of the synthetic miRNAs. For the HeLa and A549 cells, the media was changed 24 hrs after transfection. 72 hours post-transfection, cell viability was estimated by one of two methods:

(1) ViaCount Flex Reagent (Guava), which includes a dye that can only enter dead cells and that fluoresces when it interacts with DNA, was added to each well and fluorescence was quantified using the Guava PCA-96 according to the manufacturer's instructions. The percentage of viable cells was measured by dividing the number of non-dead and non-apoptotic cells in the sample by the total number of cells in the well and multiplying by 100.

(2) Propidium iodide, a dye that fluoresces when it interacts with DNA, was added to each well. Each cell was analyzed using the TTP LabTech Cell Scanner according to the manufacturer's instructions to detect cells with staining patterns consistent with cell death or apoptosis. The percentage of viable cells was measured by dividing the number of non-dead and non-

apoptotic cells in the sample by the total number of cells in the well and multiplying by 100.

Presented in FIG. 19 are synthetic miRNAs that significantly decrease or increase viability in the various cell types that were analyzed. A comparison of the viability of jurkat and primary T-cells, which represent the leukemic and normal forms of T-cells, let-7, miR-10, miR-101, miR-17-3p, miR-19, and miR-34a severely reduced the viability of the leukemia cells without affecting the normal T-cells.

EXAMPLE 24

Synthetic miRNA library screen for miRNAs that influence apoptosis

To identify miRNAs that participate in apoptosis, an apoptosis assay was used with the miRNA inhibitor library.

Approximately 8000 cervical (HeLa), prostate (22Rv1), T-cell (Jurkat), and skin (TE354T) cells per well were transfected in triplicate with each of the more than 150 synthetic miRNAs in our library using siPORT™ *NeoFX*™ (Ambion). Media was changed after 24 hrs and cells were visually inspected under a microscope to qualitatively inspect cell death 72 hours after transfection. The cells were measured for apoptosis by measuring caspase 3 activity as follows: 1) Cells were washed once with PBS and frozen at –80°C. 2) Cells were lysed by adding 40 μl of cold lysis buffer (50 mM HEPES pH 7.2, 40 mM NaCl , 0.5% NP40, 0.5 mM EDTA) to the wells and incubated for 20 min at 4°C. 3) Add 160 μl ICE buffer (50 mM HEPES pH 7.4, 0.1% CHAPS, 0.1 mM EDTA, 10% sucrose) + 5 mM DTT containing 20 μM DEVDafc substrate. 4) Measure fluorescence increase in one hour at 400 ex, 505 em. Samples were also analyzed for cell number using a general esterase assay to normalize the caspase 3 results. FDA substrate (0.4 mg/ml fluorescein diacetate (FDA) in acetonitrile) was diluted 1:19 into dilution buffer (40 mM TrisCl pH 7.5, 20 mM NaCl, 0.5% NP-40, 0.02 mg/ml final conc). 40 μl buffer (40 mM TrisCl pH 7.5, 0.5% NP-40) was added to each sample well. Samples were incubated 10 min on ice. 160 μl of diluted FDA substrate was added to each well. Fluorescence was measured for 30 min at 37 deg (ex = 488, em = 529). The slope of fluorescence increase over time is a function of the cell number in the plate.

The impact of each miRNA on apoptosis was assessed by dividing the caspase 3 reading of each well by the average caspase 3 reading for wells transfected with a negative control (NC) miRNA.

As seen in FIG. 20, many different miRNAs were able to increase or decrease apoptosis in the four cell types that were analyzed. A few miRNAs (miR-126, miR-26a, miR-1, miR-149, and let-7g) affected apoptosis in multiple cell types suggesting that they regulate apoptosis via genes that are common in multiple cell types.

EXAMPLE 25

Synthetic miRNA library screen for miRNAs that induce transformation

Transformation is necessary for tumor formation as it overcomes the cell's natural response to stop dividing when placed in a crowded environment. To identify miRNAs that participate in transformation, a transformation assay featuring NIH3T3 cells was used with the synthetic miRNA library. NIH 3T3 cells are used in transformation assays as they lack the capacity to form colonies when plated in soft agar. Modulation of cell processes that inhibit transformation can be readily detected because they induce NIH3T3 cells to begin forming colonies when plated in soft agar.

Approximately 8000 NIH 3T3 cells were transfected in duplicate with each of the more than 150 synthetic miRNAs in our library using siPORT™ *NeoFX*™ (Ambion). Media was changed after 24 hrs and the cells were transferred to 24-well dishes containing soft agar. The soft agar limits mobility and ensures that sister cells must remain in contact following cell division. Close contact with other cells typically induces the NIH 3T3 cells to stop dividing. The total number of cells in each well was measured by taking an absorbance reading at 495 nm. The absorbance reading for each well was divided by the average absorbance reading for cells transfected with negative control miRNAs and multiplied by 100 to get the percent change in transformation. An initial screen revealed miR-10, miR-23, miR-24, miR-198, miR-192, and miR-199 as miRNAs that increased transformation relative to cells transfected with negative control. A repeat of the experiment with the initial candidates yielded the following hit as shown below:

Table 31

miRNA	%NC	%SD
198	103	2.07
192	108	5.7

199 113 5.59

EXAMPLE 26

MiRNAs that affect the efficacy of therapeutic compounds

Many compounds have been tested in clinical trials for their capacity to positively affect the outcome of patients. In some cases, these compounds meet the standards set for by the FDA and they become therapeutics. Unfortunately, very few therapeutics are 100% effective. Enhancing the activities of therapeutic compounds provides a significant opportunity within the medical industry. The two most common methods that are used to enhance therapeutics are modifying the chemical structure of the compounds or using multiple therapeutic compounds simultaneously. Whether it would be beneficial to introduce miRNAs in advance of adding compounds that are known to significantly reduce the viability of cancer cells was evaluated. One of the anti-cancer compounds that was introduced was TRAIL, a compound that binds at least two different receptors and activates the apoptosis pathway to induce cell death primarily in cancer cells. The second compound that was tested in combination with synthetic miRNAs was etoposide, a topoisomerase II inhibitor that activates the apoptosis pathway of cancer and normal cells alike by reducing the repair of DNA damage within the cells.

Approximately 8000 cervical (HeLa) and lung (A549, HTB-57, and CRL-5826) cells per well were transfected in triplicate with synthetic miRNAs from our library using siPORT™ *NeoFX*™ (Ambion). Media was changed after 24 hrs and etoposide and TRAIL were introduced at a final concentration of approximately 25 μM after 48 hours. The cells were visually inspected under a microscope to qualitatively inspect cell death 64 hours after transfection.

The cells treated with etoposide were measured for apoptosis by measuring caspase 3 activity as follows: 1) Cells were washed once with PBS and frozen at –80°C. 2) Cells were lysed by adding 40 μl of cold lysis buffer (50 mM HEPES pH 7.2, 40 mM NaCl , 0.5% NP40, 0.5 mM EDTA) to the wells and incubated for 20 min at 4°C. 3) Add 160 μl ICE buffer (50 mM HEPES pH 7.4, 0.1% CHAPS, 0.1 mM EDTA, 10% sucrose) + 5 mM DTT containing 20 μM DEVDafc substrate. 4) Measure fluorescence increase in one hour at 400 ex, 505 em. Samples were also analyzed for cell number using a general esterase assay to normalize the caspase 3 results. FDA substrate (0.4 mg/ml fluorescein diacetate (FDA) in acetonitrile) was diluted 1:19 into dilution buffer (40 mM TrisCl pH 7.5, 20 mM NaCl, 0.5% NP-40, 0.02 mg/ml final conc). 40 µl buffer (40 mM TrisCl pH 7.5, 0.5% NP-40) was added to each sample well. Samples were incubated 10 min on ice. 160 µl of diluted FDA substrate was added to each well. Fluorescence was measured for 30 min at 37 deg (ex = 488, em = 529). The slope of fluorescence increase over time is a function of the cell number in the plate.

The cells treated with TRAIL were assessed for cell viability by adding alamar blue each well and analyzing fluorescence using a plate reader. Alamar blue is a substrate for a metabolic enzyme in cells and the reaction product is fluorescent. The fluorescence in each well correlates with the total number of cells in each well.

The effect of each miRNA on the treatments was measured by dividing the caspase 3 or alamar blue reading of the cells transfected with miRNAs and treated with TRAIL or etoposide by the same readings for cells that were only transfected with the miRNAs. The change in caspase 3 activity or alamar blue staining for each miRNA was then divided by the differences observed for two negative control miRNAs and multiplied by 100 to calculate the relative effect induced by the combination of each miRNA and the therapeutic compound. These values are listed as %NC in Figure G.

As shown in FIG. 21, a number of miRNAs significantly increased the capacity of the two therapeutic compounds to induce cell death in the cancer cells that were treated. Interestingly, mir-292-3p, mir-132, mir-124, and mir-28 all worked extremely well in combination with both TRAIL and etoposide.

EXAMPLE 27

Synthetic miRNA library screen for miRNAs that affect cell cycle

The adult human body consists of about 50-100 trillion cells. Each day, several billion of these cells divide in two to replace the billions of cells that die and are removed. In the course of an average lifetime, this adds up to an astronomical number of cell divisions, most of which go perfectly well. Errors do occur, however, and if they are not corrected they may lead to cancer. Cell growth and division are normally controlled by an intricate system of checks and balances. But occasionally a cell will start to proliferate wildly, dividing again and again and defying all normal restraints on its growth. That is the beginning of most common forms of cancer.

Approximately 8000 cervical (HeLa) and 4000 skin (BJ) cells per well were transfected in triplicate with each of the more than 150 synthetic miRNAs in our library. HeLa cells were transfected using siPORT™ *NeoFX*™ (Ambion) and BJ cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. 24 hours post-transfection, half of the cells from each well were removed to fresh medium. 72 hrs post-transfection, the cells were fixed with 4% paraformaldehyde at a final concentration of 2%. The fixed cells were stained with propidium iodide (TTP LabTech protocol) and assessed using the TTP LabTech cell scanner. Propidium iodide stains DNA and the relative DNA content in a cell corresponds with its position in the cell cycle. The cell scanner measured propidium iodide staining in each cell and assigned its position in the cell cycle. The percentage of cells in each stage of cell cycle was calculated and compared to cells transfected with negative control synthetic miRNAs. The relative change in cells in each stage was calculated for each miRNA that was used. Those synthetic miRNAs that induced a significant shift toward or away from a specific stage of cell cycle are listed below. These represent miRNAs that regulate key points in the cell cycle and offer key intervention points for cancer-related therapeutic development.

As seen in FIG. 22, many different miRNAs significantly altered the percentage of cells in the various stages of cell cycle in the two cell types that were analyzed.

EXAMPLE 28

Synthetic miRNA library screen for miRNAs that influence ERK activity

In order for cancer cells to proliferate they must subvert both the machinery that controls the cell division cycle and the process of programmed cell death (apoptosis). This is frequently achieved by mutation of specific proto-oncogenes such as *Ras* or tumor suppressors such as *p53*. The Ras-family of membrane associated GTPases transmit signals into the interior of the cell by the activation of a number of cytosolic signal transduction pathways such as the Raf>MEK>ERK MAP kinase signaling pathway. Disregulation of the Ras/Raf/MEK/ERK pathway plays a major role in cancer pathogenesis (reviewed in Meijer *et al.*).

To identify miRNAs that affect ERK activation, HeLa cells were transfected in a 96-well plate format with 160 different synthetic miRNAs. Prior to transfection, the HeLa cells were trypsinized to remove adherent cells and diluted in normal growth medium to 10^5 cells/mL. 0.5 µl of siPort NeoFX in 9.5 µl of Optimem I medium was added to the cells and incubated for 10 minutes at room temp (10 µL for each sample). miRNAs were rehydrated with 10 µl of diluted siPORT NeoFX. The samples were incubated at 37 °C and then the transfected samples were evaluated 72 hours after transfection.

The controls for ERK activation were performed by depriving the wells of a phosphate source for detection of ERK phosphorylation. 100 µl of serum-free media (DMEM) to 37°C was added per well and the cells were incubated for 4 hours at 37 °C to attain basal phosphorylation levels. For the positive control wells, serum-free media was aspirated from wells and 100 µL of 100 ng/mL EGF was added before incubating the cells for 7.5 minutes at 37 °C.

Media from all wells was removed by aspiration and the cells were immediately fixed in 150 µL of 3.7% Formaldehyde in 1X PBS for 20 minutes at room temp with no shaking. Fixing solution was removed to an appropriate waste container. The fixed cells were washed three times with 1X PBS. The wells were then washed three times with 200 µL of 1X PBS containing 0.1% Triton X-100 for 5 minutes per wash, with shaking at room temp.

Cells were blocked by adding 150 µL of Li-COR Odyssey Blocking Buffer™ to each well. The solution was moved carefully by pipetting down the sides of the wells to avoid detaching the cells. Blocking was for 90 minutes at room temp with moderate shaking on a rotator and the two primary antibodies were added to a tube containing Odyssey Blocking Buffer. The primary antibody was incubated for 2 hours with gentle shaking at room temp (Phosho-ERK (Rabbit, 1:100 dilution; Cell Signaling Technology 9101). Total ERK2 (Mouse; 1:75 dilution; Santa Cruz Biotechnology SC-1647)). The wells were washed three times with 1X PBS + 0.1% Tween-20 for 5 minutes at room temp with gentle shaking, using a generous amount of buffer. The fluorescently labeled secondary antibody was diluted in Odyssey Blocking Buffer (Goat anti-rabbit Alexa Fluor 680™ (1:200 dilution; Molecular Probes) Goat anti-mouse IRDye 800CW™ (1:800 diution; Rockland Immunochemicals)). The antibody solutions were mixed well and 50 µL of the secondary antibody solution was added to each well. The antibody solution was incubated for 60 minutes with gentle shaking at room temp. The plate was washed three times with 1X PBS + 0.1% Tween-20 for 5 minutes at room temp with gentle shaking, using a generous amount of buffer. After a final wash, wash solution was completely removed from wells. The plates were scanned with the Odyssey Infrared Imaging System (700 nm detection for Alexa Fluor 680 antibody and 800 nm detection for IRDye 800CW antibody).

Table 32

miRNAs That Activate ERK

miRNA	% NC	%StDev
let-7	250	25
mir-125a	252	27
mir-134	211	13
mir-148	264	44
mir-152	266	36
mir-19a	242	31
mir-206	256	56
mir-207	224	3
mir-210	292	38
mir-212	231	24
mir-216	219	26
mir-217	273	27
mir-218	313	23
mir-223	264	40
mir-25	239	45
mir-294	236	25
mir-295	221	14
mir-301	261	62
mir-328	259	46
mir-329	243	5
mir-339	214	42
mir-370	221	23
mir-372	211	6
mir-96	214	57
Positive Control	245	11

EXAMPLE 29

Synthetic miRNA library screen for miRNAs that influence hTert expression

Telomerase is a complex of proteins and RNA that maintains the ends of chromosomes by appending telomeres. With rare exceptions, terminally differentiated cells lack active telomerase. One of the exceptions is cancer cells.

More than 90% of human cancer samples have active telomerase (reviewed in Dong *et al.*, 2005). The hTert gene encodes the catalytic domain of telomerase. The expression of hTert correlates with telomerase activity in cells making it a good surrogate for telomerase activity. We have developed and used an RT-PCR based assay for monitoring hTert mRNA expression in telomerase negative cells to identify miRNAs that participate in the regulation of telomerase. The miRNAs that regulate telomerase activity represent intervention points for cancer therapeutics.

BJ cells are normal foreskin fibroblasts that lack hTert mRNA and telomerase activity. BJ cells were trypsinized and diluted to 13,000 cells/ml in normal growth media. 0.3 μl of lipofectamine 2000 agent was diluted into 40 μl of OPTI-MEM and incubated for five minutes. The diluted transfection reagent was added to the wells of 96-well plates that contained 151 synthetic miRNAs as well as two different negative control synthetic miRNAs. Each well housed a different synthetic miRNA. The synthetic miRNAs and transfection agent were incubated for 15 minutes at room temperature and then 200 μl (2,600 cells) were added on top of the lipid/miRNA complex. Cells were placed in an incubator and RNA was isolated 72 hours later. RNA was isolated from the cells in each well using RNAqueous™-MagMAX96 Total RNA Isolation kit (Cat#1830) standard protocol (lyse cells in wells). Reverse transcription was done using the RETROscript reaction by adding 11 ul of total RNA (20-100 ng/μl) to 1 μl of random decamers and incubated in 70°C water bath for 3 minutes then place on ice. Next, 8 μl of the cocktail containing Nuc-free water 3.8 μl, 10X Reverse Transcription buffer 2.0 μl, 2.5 mM dNTPs 2.0 μl, RNase Inhibitor Protein (40 U/μl), 0.1 μl MMLV-RT (100 U/μl), and incubated at 42°C for 1 hour, then 92°C for 10 minutes.

Real time PCR reactions were assembled to quantify hTert mRNA and 18S rRNA in each of the samples. Nuclease-free water, 10X Complete PCR buffer/SYBR, 25 mM MgCl2, 2.5 mM dNTPs, 50X ROX, 18S- or hTert-specific primers (for & rev mix 3 μM), cDNA from the various samples, and Super taq polymerasewere placed into a PCR tube. The reaction was heated to 95°C for 5 minutes and then subjected to 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, 72°C for 30 seconds. The amplification products were monitored using the ABI 7600 (Applied Biosystems). BJ cells ordinarily fail to yield amplification products with the hTert primers. Those miRNA-transfected samples that yielded a hTert PCR product were also analyzed for 18S rRNA lavels to ensure that there were not significantlyt more cells in the samples that might have contributed to the amount of hTert in the samples.

The hTert mRNA was detected in duplicate transfections of each of the miRNAs listed below. These miRNAs presumably affect pathways that regulate the expression of the hTert gene. Over-expression of any of these miRNAs might contribute to cancer by activating telomerase. Regulating the activities of these miRNAs in cancer cells could limit their transformation and overcome oncogenesis.

Table 33

hTert miRNA Activators

miRNA	Log(2) hTert Expression
mir-147	3.14
mir-195	4.25
miR-21	1.55
mir-24	4.68
mir-26a	4.35
mir-301	4.14
mir-368	5.30
mir-371	2.43

The telomerase activity screen was repeated using a series of siRNAs targeting kinases, phosphatases, GPCRs, transcription factors, and assorted other genes. Targeting the genes below with siRNAs resulted in increased hTert expression. Interestingly, many of these genes are predicted to be targets for the miRNAs that we found to be hTert regulators (see table below).

Table 34

hTert Gene Activators

Gene	Log(2) hTert Expression
ACOX1	3.44
AKT1	1.80
APAF1	3.40
COX-5B	2.78
COX6	2.28
COX7B	3.95
CPOX	4.66
DUOX2	3.80
GPX1	1.85
GPX2	2.56
GPX4	3.17
LPO	3.37
MAPK1	3.07

MAPK4	3.61
MTCO1	1.58
NOX3	2.30
NOX5	2.54
PAOX	1.72
PPOX	2.09
PRKCA	2.24
PRKCD	4.39
TNFRSF6	2.25

EXAMPLE 30

Effect of miRNA primary sequence on function

Many miRNAs appear to be very closely related to others based on their primary sequences. For instance, let-7a is a member of the let-7 gene family, which includes 7 unique genes within the human genome. The let-7 genes encode miRNAs that vary by as little as a single nucleotide and as many as four nucleotides. In our synthetic miRNA and miRNA inhibitor libraries, we have five different human let-7 miRNAs. These miRNAs have been used in many different cell types in screens designed to identify miRNAs involved in a variety of different cellular processes. In many of the screens, the various let-7 miRNAs generate similar phenotypes. FIG. 223 provides two examples wherein all of the let-7 family members yield similar responses. In contrast, there are some screens wherein the various let-7 family miRNAs yield significantly different results (FIG. 23).

EXAMPLE 31

Synthetic miRNA library screen for miRNAs that influence inflammation

Inflammation is the body's natural protective response to an injury or infection. It is designed to hyper-stimulate biological pathways that initiate tissue repair or attack invading pathogens. This response is a delicate balance of both pro- and anti-inflammatory genes and their proteins. If the inflammatory response is maintained too long it can lead to tissue destruction, organ failure or inflammatory diseases such as Rheumatoid arthritis, Psoriasis, Asthma, Inflammatory bowel disease (Crohn's disease and related conditions), Multiple Sclerosis, coronary obstructive pulmonary disease (COPD), Allergic rhinitis (hay fever), and Cardiovascular disease.

Stat3 is the subject of intense scientific investigation, because it's known to be an important transcription factor that turns on genes required for the cell division, induction and suppression of apoptosis, and cell motility. Many STAT3 target genes are known, including those encoding the anti-apoptotic proteins Bcl-xl, Mcl-1, and Bcl-2, the proliferation-associated proteins Cyclin D1 and Myc, and the pro-angiogenic factor VEGF. The inflammatory disease psoriasis is characterized by lesions, which contain epidermal keratinocytes that express high levels of activated Stat3. Stat3 has also recently been discovered to play an important role as an anti-inflammatory regulator. In normal mice, the immune system is initially upregulated in response to bacterial protein challenge creating systemic inflammation followed by down regulation of the initiating factors. Mice with a deletional mutation for Stat3-beta lacked the ability to down regulate the initial inflammatory reaction after bacterial protein challenge which lead to irreversible damage to the animals' own tissues and finally to animal death.

A stat3 response assay was used to identify miRNAs that regulate cellular inflammatory response. The stable Stat3-luciferase reporter cell line from Panomics, which contains a chromosomal integration of a luciferase reporter construct regulated by 3 copies of the Stat1 response element was used for this purpose. The chemical agent Phorbol-12-myristate 13 acetate (PMA) is known to induce an inflammatory response in exposed cells and was used to stimulate inflammation in this experiment. These cells were transfected in triplicate with each of the more than 206 synthetic miRNAs in our library using siPORT™ *NeoFX*™ (Ambion) at a plating density of approximately 6000 cells/well of 96 well plate.. The media was changed 24h post transfection and exposed to 100nM PMA for 6 hours starting at 67 hours post transfection. The cells were assayed for changes in total cell number by alamarBlue as previously described and finally harvested at 72 hours post initial transfection. A luciferase assay was performed on all sample lysates to measure Stat3 responsiveness to the procedure. The data was normalized to total cell number using the alamar Blue data and compared to cells transfected with a negative control miRNA that underwent the same procedure.

The following miRNA were able to reduce the ability of PMA to stimulate Stat3.

	% of NC	% STDEV
mir-93	34	74
mir-100	13	10
mir-134	50	18
mir-99a	38	96
mir-103	38	40
mir-128	49	115
mir-129	44	112
mir-181b	11	21
mir-193	42	92
mir-197	36	78
mir-212	42	92
mir-218	38	84
mir-219	39	86
mir-302	40	87
mir-323	22	49
mir-324-3p	29	63
mir-325	29	63
mir-330	21	47
mir-331	39	86
mir-340	34	75
mmu-mir-350	11	22
mir-425	24	49
mir-491	25	49
mir-518f	26	52
mir-520a*	28	55

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CLAIMS:

1. A synthetic RNA molecule comprising:

a) a first 17-25 residue oligonucleotide comprising a sequence that is 80-100% identical to a mature microRNA sequence;

b) a second 17-25 residue oligonucleotide comprising a sequence that is 60-100% identical to a sequence complementary to the mature microRNA sequence; and

c) at least one of

i) a replacement group for the phosphate or the hydroxyl of the nucleotide at the 5' end of the second oligonucleotide,

ii) one or more sugar modifications in the first or last 1-6 residues of the second oligonucleotide, and

iii) a noncomplementarity between one or more nucleotides in the last 1-5 residues at the 3' end of the second oligonucleotide and the corresponding nucleotides of the first oligonucleotide.

2. A synthetic RNA molecule comprising:

a) a first 17-25 residue oligonucleotide comprising a sequence that is 80-100% identical to a mature microRNA sequence;

b) a second 17-25 residue oligonucleotide comprising a sequence that is 60-100% identical to a sequence complementary to the mature microRNA sequence; and

c) at least one modification to the second oligonucleotide that enhances the uptake of the first oligonucleotide.

3. The synthetic RNA molecule of claim 1 or 2, comprising a replacement group for the phosphate or the hydroxyl of the nucleotide at the 5' end of the second oligonucleotide.

4. The synthetic RNA molecule of claim 3, wherein the replacement group is biotin, an amine group, a lower alkylamine group, an acetyl group, 2'O-Me, DMTO, fluoroscein, a thiol, or acridine.

5. The synthetic RNA molecule of claim 1 or 2, wherein the phosphate of the nucleotide at the 5' end of the second oligonucleotide is replaced.

6. The synthetic RNA molecule of claim 5, wherein the replacement group is a lower alkylamine group.

7. The synthetic RNA molecule of claim 1 or 2, wherein the hydroxyl of the nucleotide at the 5' end of the second oligonucleotide is replaced.

8. The synthetic RNA molecule of any one of claims 1-7, comprising one or more sugar modifications in the first or last 1-6 residues of the second oligonucleotide.

9. The synthetic RNA molecule of any one of claims 1-7, comprising one or more sugar modifications in the first 1-6 residues of the second oligonucleotide.

10. The synthetic RNA molecule of any one of claims 1-7 or 9, comprising one or more sugar modifications in the last 1-6 residues of the second oligonucleotide.

11. The synthetic RNA molecule of any one of claims 1-7, comprising one or more sugar modifications in the first or last 2 to 4 residues of the second oligonucleotide.

12. The synthetic RNA molecule of any one of claims 1-7 or 11, comprising one or more sugar modifications in the first 2 to 4 residues of the second oligonucleotide.

13. The synthetic RNA molecule of any one of claims 8-12, wherein the sugar modification is a 2'O-Me modification.

14. The synthetic RNA molecule of any one of claims 1-13, comprising a noncomplementarity between one nucleotide in the last 1-5 residues at the 3' end of the second oligonucleotide and the corresponding nucleotides of the first oligonucleotide.

15. The synthetic RNA molecule of any one of claims 1-13, comprising a noncomplementarity between two or more nucleotides in the last 1-5 residues at the 3' end of the second oligonucleotide and the corresponding nucleotides of the first oligonucleotide.

16. The synthetic RNA molecule of any one of claims 1-15, comprising two of i), ii), and iii).

17. The synthetic RNA molecule of any one of claims 1-15, comprising i), ii), and iii).

18. The synthetic RNA molecule of any one of claims 1-17, wherein the mature microRNA sequence is a mature miR-1, preferably miR-1-1 or miR-1-2; miR-7, preferably miR-7-1, miR-7-2, or miR-7-3; miR-10, preferably miR-10a or miR-10b; miR-15, preferably miR-15a or miR-15b; miR-16, preferably miR-16-1 or miR-16-2; miR-21; miR-24, preferably miR-24-1 or miR-24-2; miR-26, preferably miR-26a-1, miR-26b, or miR-26a-2; miR-28; miR-29, preferably miR-29b-1, miR-29c, or miR-29b-2; miR-31; miR-34 sequence, preferably a mature miR-34a, miR-34b, or miR-34c sequence; miR-92, preferably miR-92-1 or miR-92-2; miR-96; miR-101, preferably miR-101-1 or miR-101-2; miR-105, preferably miR-105-1 or miR-105-2; miR-124, preferably miR-124 is miR-124a-1, 124a-2, or 124a-3; miR-126; miR-133, preferably miR-133a-1, miR-133b, or miR-133a-2; miR-142; miR-143; miR-147; miR-155; miR-181, preferably miR-181a, miR-181b-1, or miR-181b-2; miR-192; miR-193; miR-194, preferably miR-194-1 or miR-194-2; miR-200, preferably miR-200a, miR-200b, or miR-200c; miR-205; miR-206; miR-215; miR-221; miR346; miR-449 sequence; or let-7, preferably let-7a-1, let7-a-2, let7-a-3, let-7b, let-7c, let-7c-1, let-7c-2, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, or let-7 sequence.

19. The synthetic RNA molecule of any one of claims 1-18, wherein the first oligonucleotide is 85, 90, 95, or 100% identical to the mature microRNA sequence.

20. The synthetic RNA molecule of any one of claims 1-19, wherein the second oligonucleotide is at least 65, 70, 75, 80, 85, 90, 95, or 100 % identical to the sequence complementary to the mature microRNA sequence.

21. A pharmaceutical composition comprising the synthetic RNA molecule of any one of claims 1-20.

22. The pharmaceutical composition of claim 21, wherein the pharmaceutical composition comprises a sterile aqueous solution.

23. The pharmaceutical composition of claim 21 or 22, wherein the synthetic RNA molecule is in a liposomal pharmaceutical delivery system.

24. The pharmaceutical composition of any one of claims 21-23, wherein the pharmaceutical composition comprises an antibacterial or antifungal agent.

25. The pharmaceutical composition any one of claims 21-24, wherein the pharmaceutical composition comprises at least about 0.1% of the synthetic RNA molecule, between about 2% to about 75% by weight of the synthetic RNA molecule, or between about 25% to about 60% by weight of the synthetic RNA molecule.

26. The pharmaceutical composition of any one of claims 21-25, wherein the synthetic RNA molecule is at least about 95, 96, 97, 98, 99, or 100% pure.

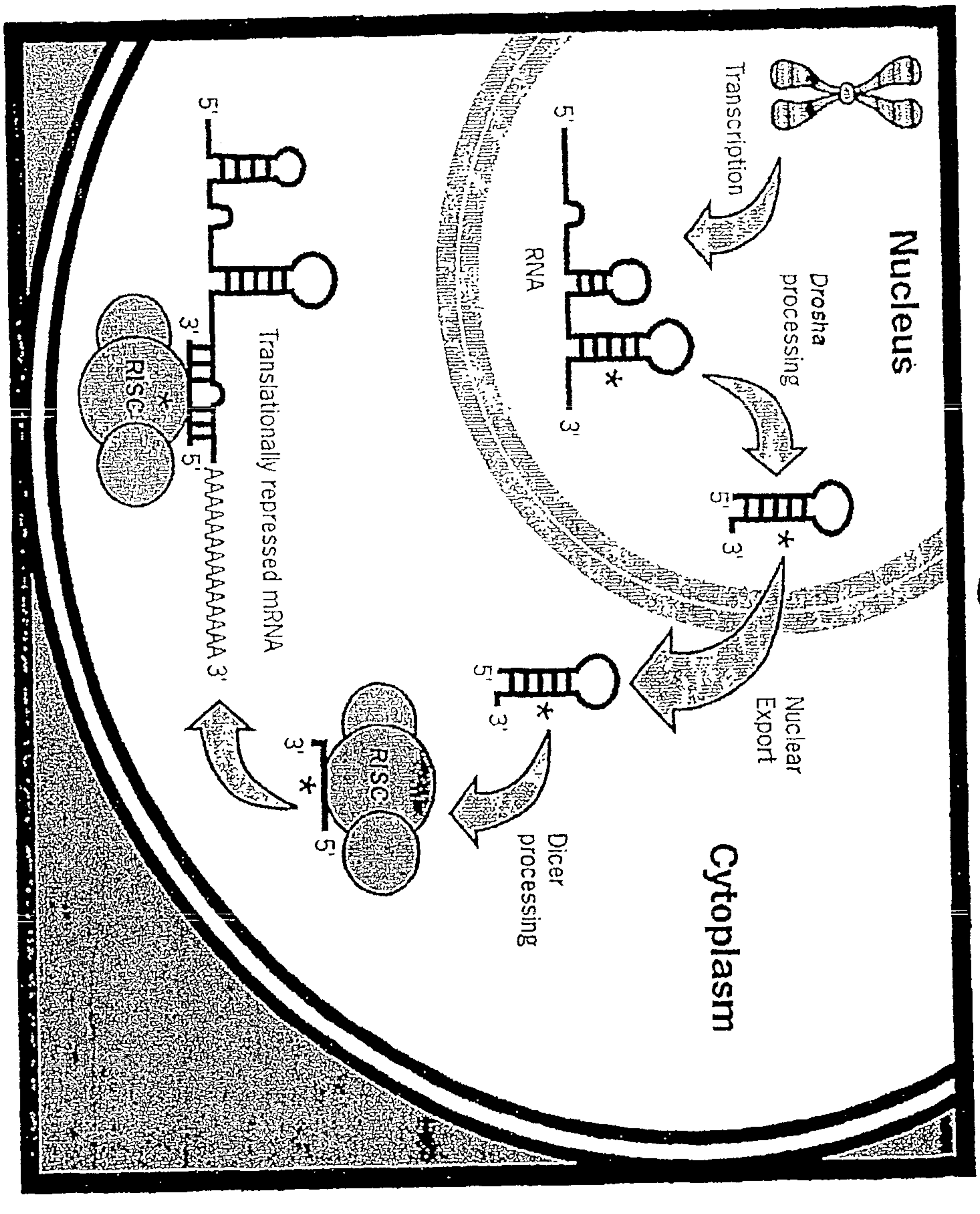
27. The pharmaceutical composition of any one of claims 21-26, wherein the pharmaceutical composition is aliquoted in a vial, test tube, flask, bottle, syringe, or container.

28. The pharmaceutical composition of any one of claims 21-27, wherein the pharmaceutical composition comprises a dose of about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 5 mg/kg body weight, about 10 mg/kg body

weight, about 50 mg/kg body weight, about 100 mg/kg body weight, about 200 mg/kg body weight, about 350 mg/kg body weight, about 500 mg/kg body weight, about 1000 mg/kg body weight, about 5 mg/kg body weight to about 100 mg/kg body weight, or about 5 microgram/kg/body weight to about 500 mg/kg body weight.

29. The pharmaceutical composition of any one of claims 21-28, wherein the pharmaceutical composition is formulated for topical, intravenous, injection, transdermal, transmucosal, inhalation, oral, or pulmonary administration.

30. A use of the pharmaceutical composition of any one of claims 21-29 for treatment of a disease or a condition in a subject.



Nucleus
Cytoplasm
Transcription
RNA
Drosha processing
Nuclear Export
Dicer processing
RISC
RISC
Translationally repressed mRNA
AAAAAAAAAAAAAA 3'
5'
3'

Fig. 1

Fig. 2

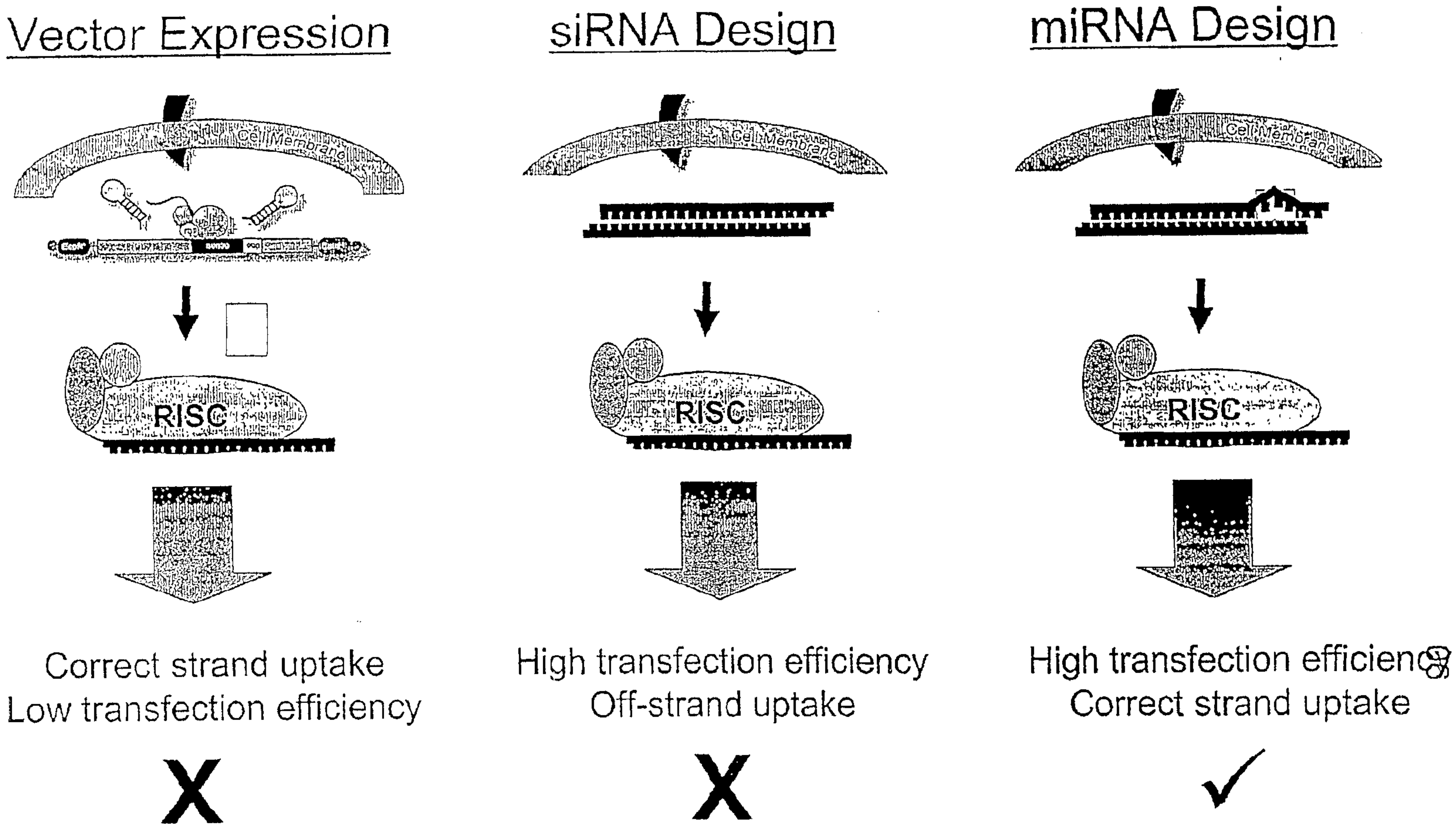
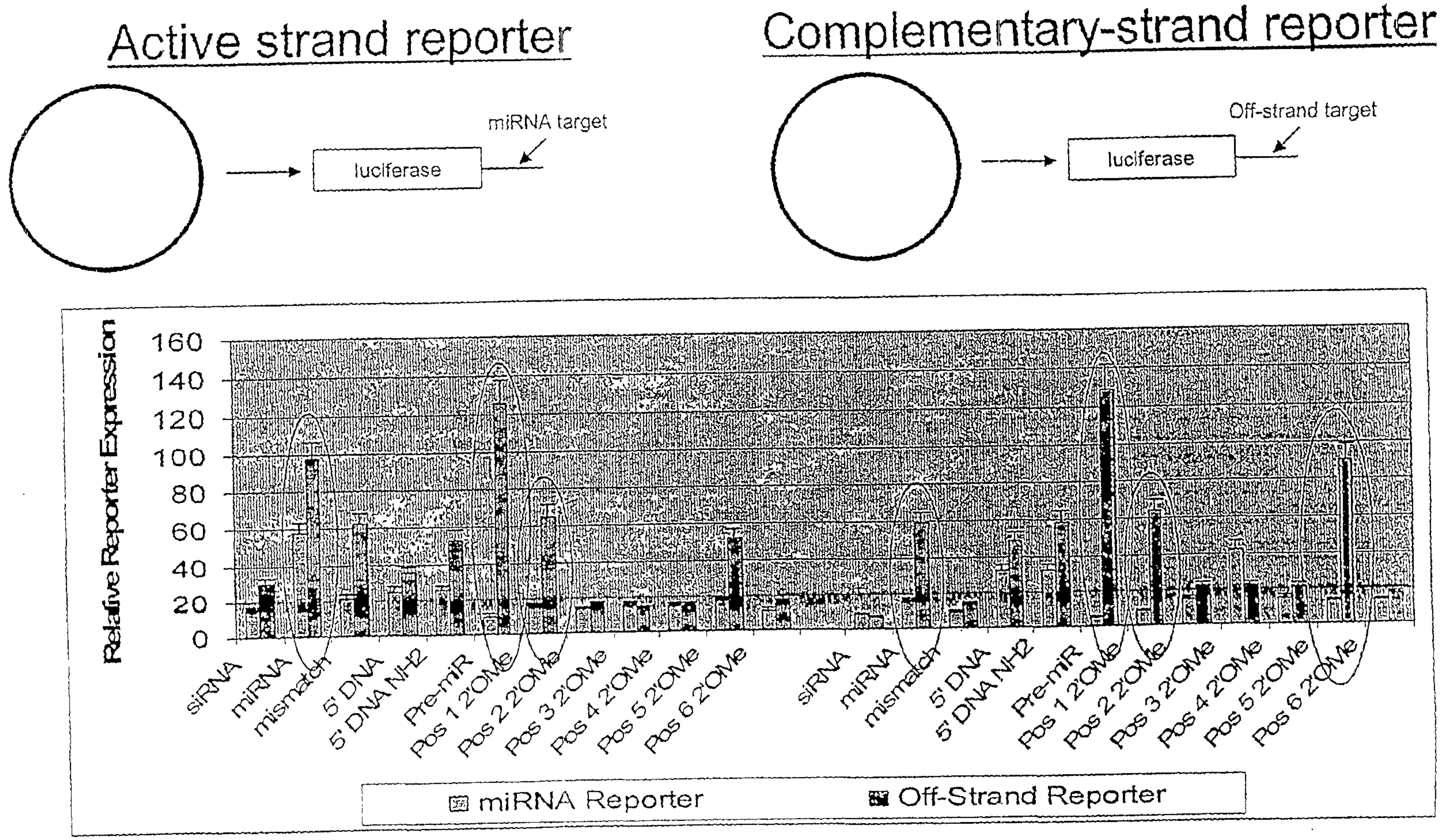

Vector Expression
siRNA Design
miRNA Design
Cell Membrane
Cell Membrane
Cell Membrane
RISC
RISC
RISC
Correct strand uptake
Low transfection efficiency
X
High transfection efficiency
Off-strand uptake
X
High transfection efficiency
Correct strand uptake
✓

Fig. 3


Active strand reporter
luciferase
miRNA target
Complementary-strand reporter
luciferase
Off-strand target
Relative Reporter Expression
0
20
40
60
80
100
120
140
160
siRNA
miRNA
mismatch
5' DNA
5' DNA NH2
Pre-miR
Pos 1 2'OMe
Pos 2 2'OMe
Pos 3 2'OMe
Pos 4 2'OMe
Pos 5 2'OMe
Pos 6 2'OMe
siRNA
miRNA
mismatch
5' DNA
5' DNA NH2
Pre-miR
Pos 1 2'OMe
Pos 2 2'OMe
Pos 3 2'OMe
Pos 4 2'OMe
Pos 5 2'OMe
Pos 6 2'OMe
miRNA Reporter
Off-Strand Reporter

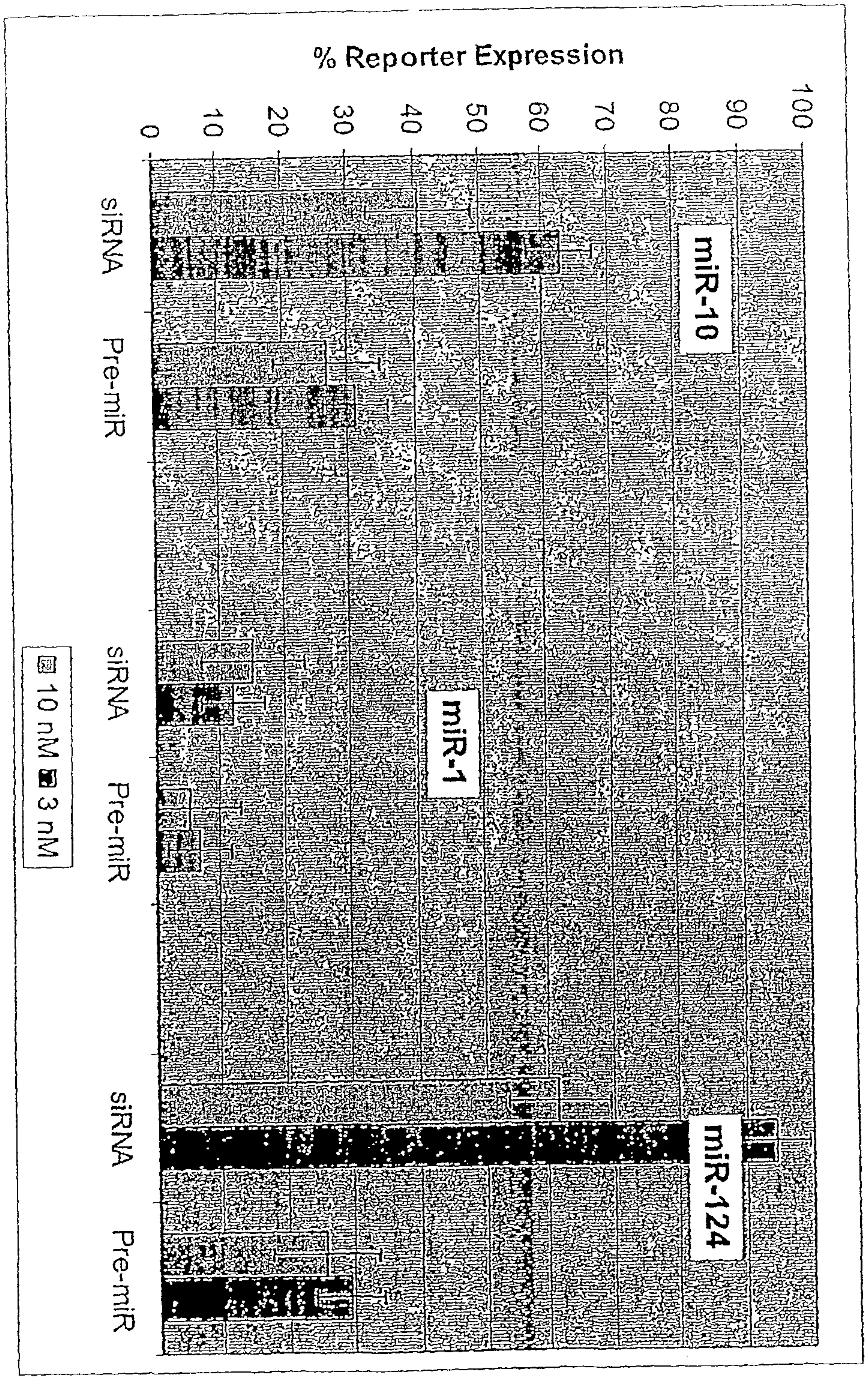
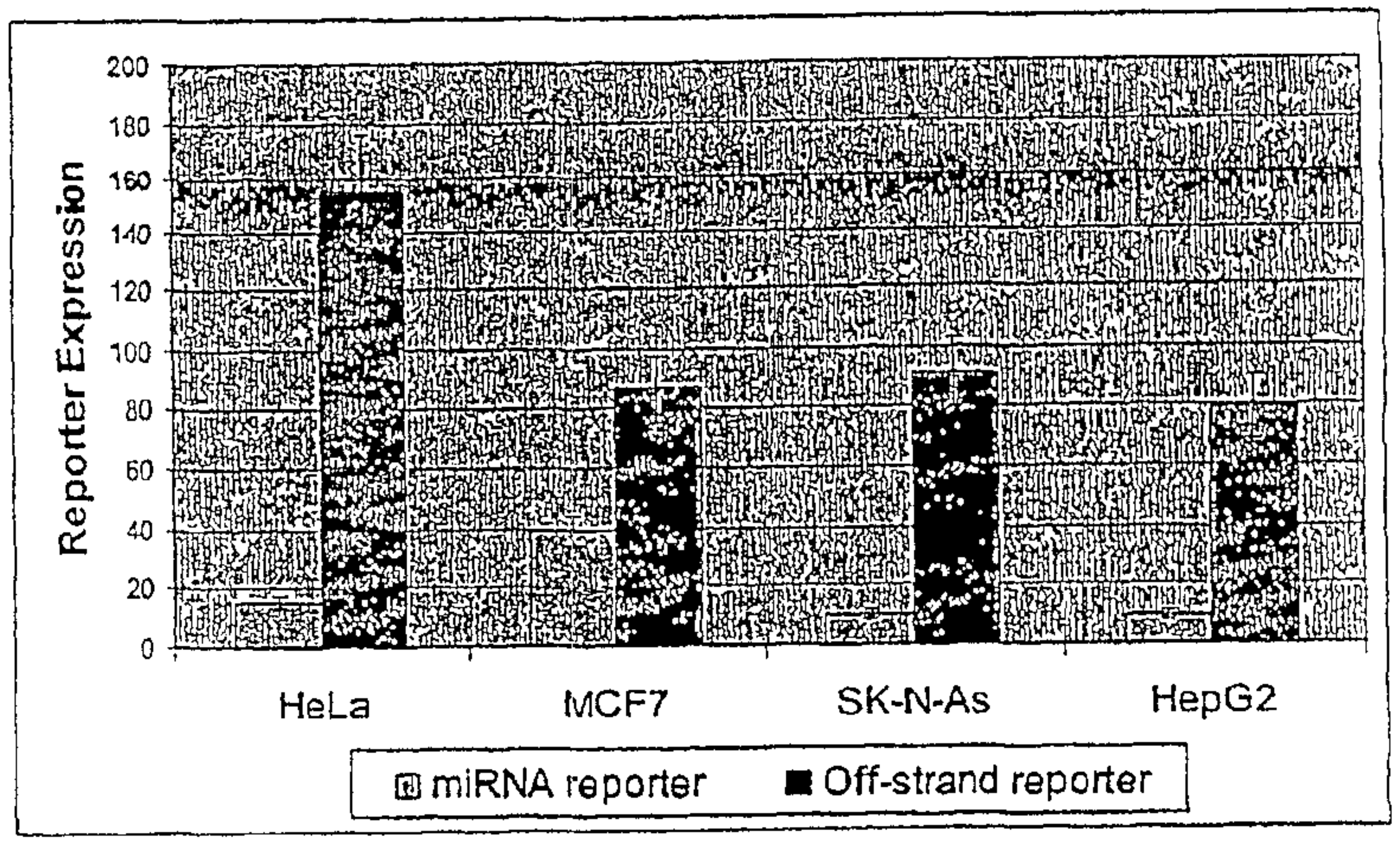
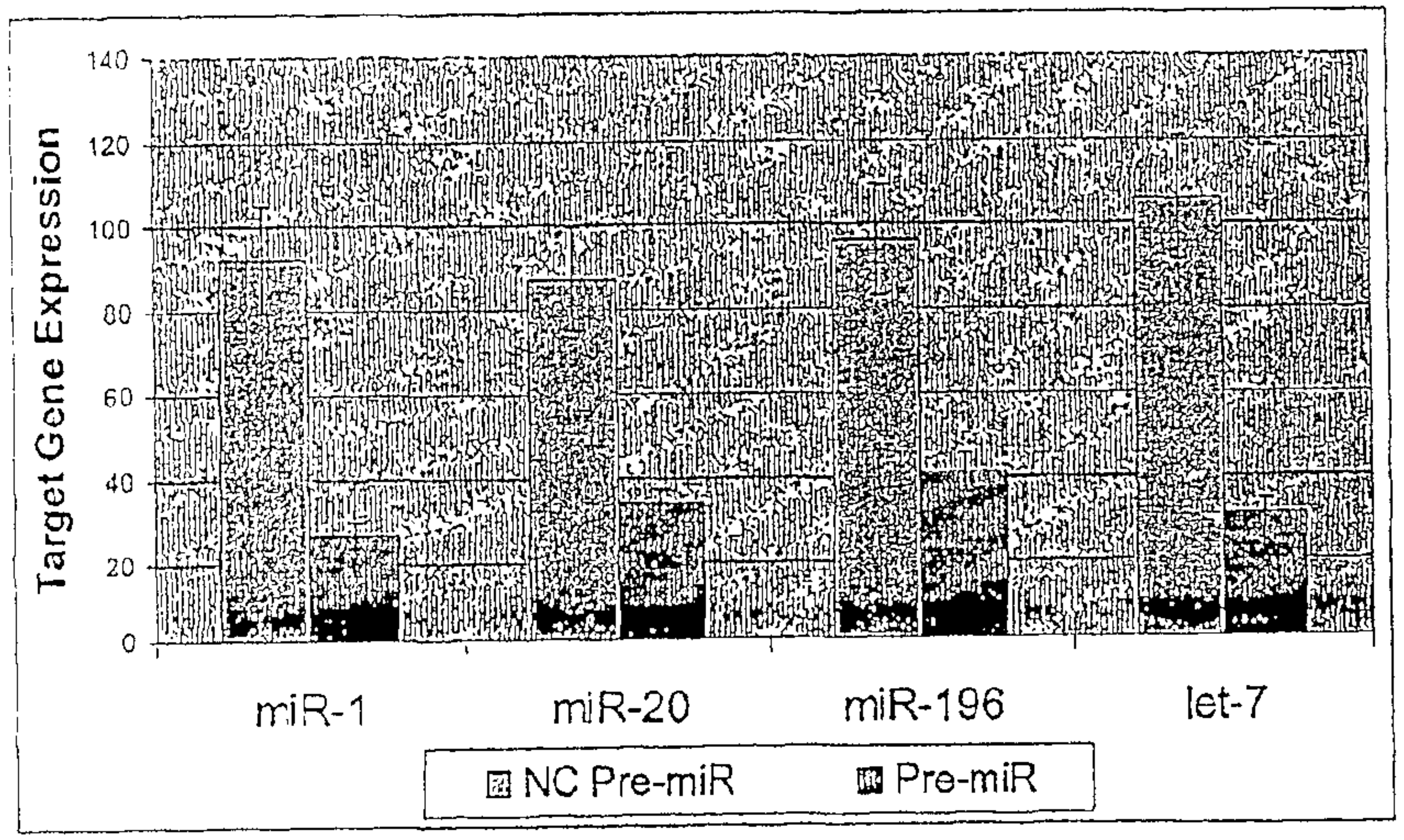

% Reporter Expression
0
10
20
30
40
50
60
70
80
90
100
miR-10
miR-1
miR-124
siRNA
Pre-miR
siRNA
Pre-miR
siRNA
Pre-miR
10 nM
3 nM

Fig. 4

Fig. 5

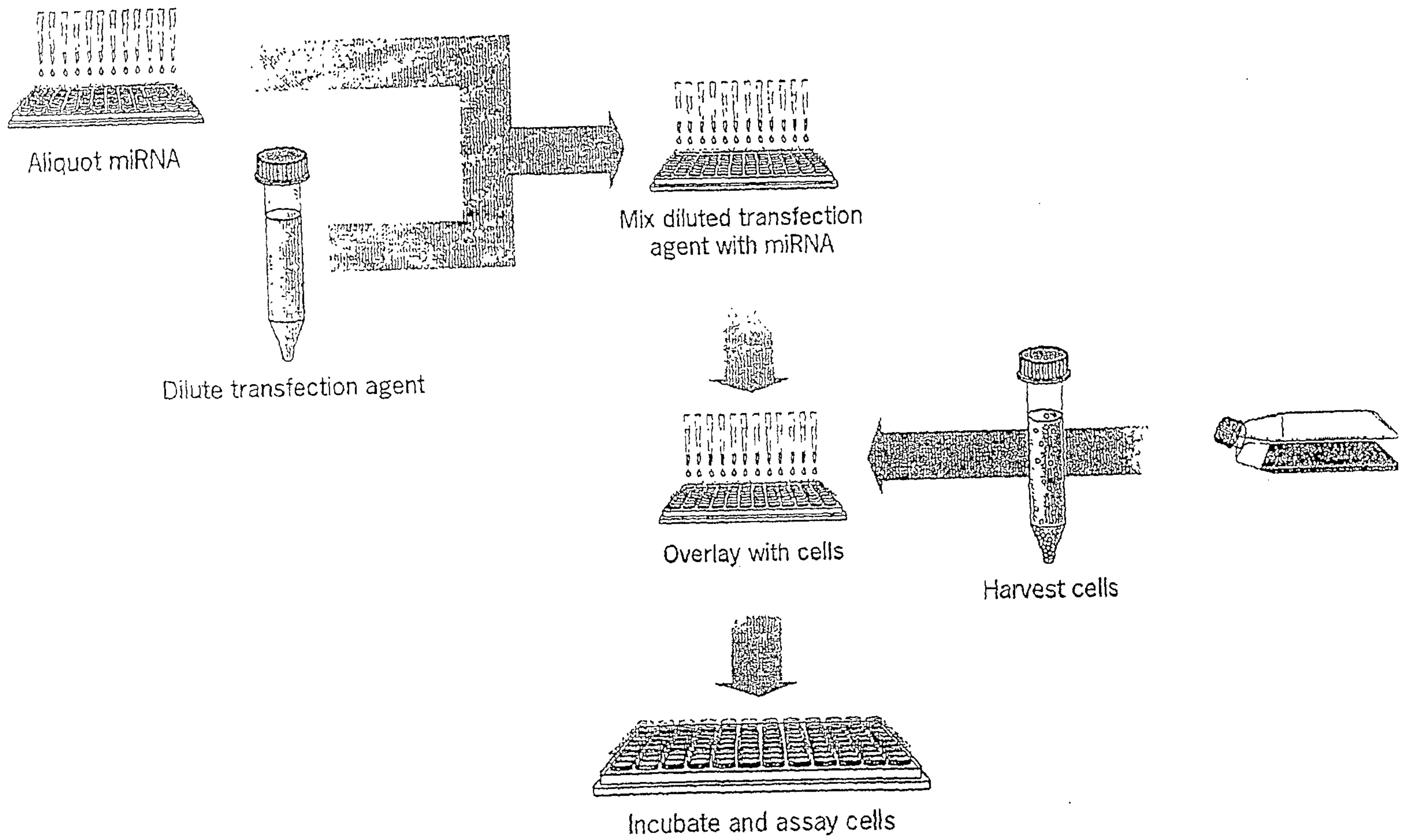
Synthetic miRNAs in multiple cell types





Effect of Synthetic miRNAs on endogenous targets

Fig. 6


Aliquot miRNA
Dilute transfection agent
Mix diluted transfection agent with miRNA
Overlay with cells
Harvest cells
Incubate and assay cells

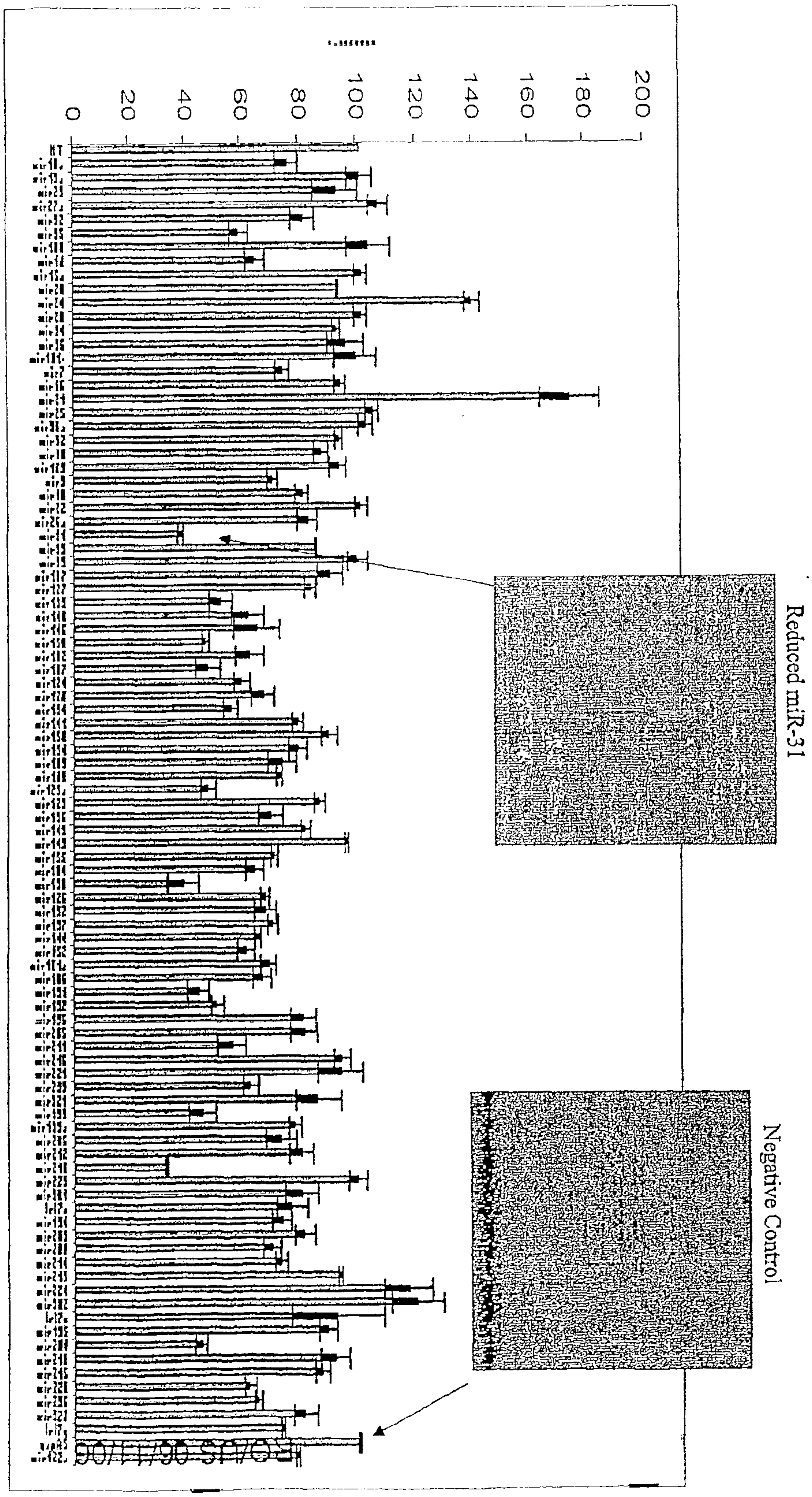
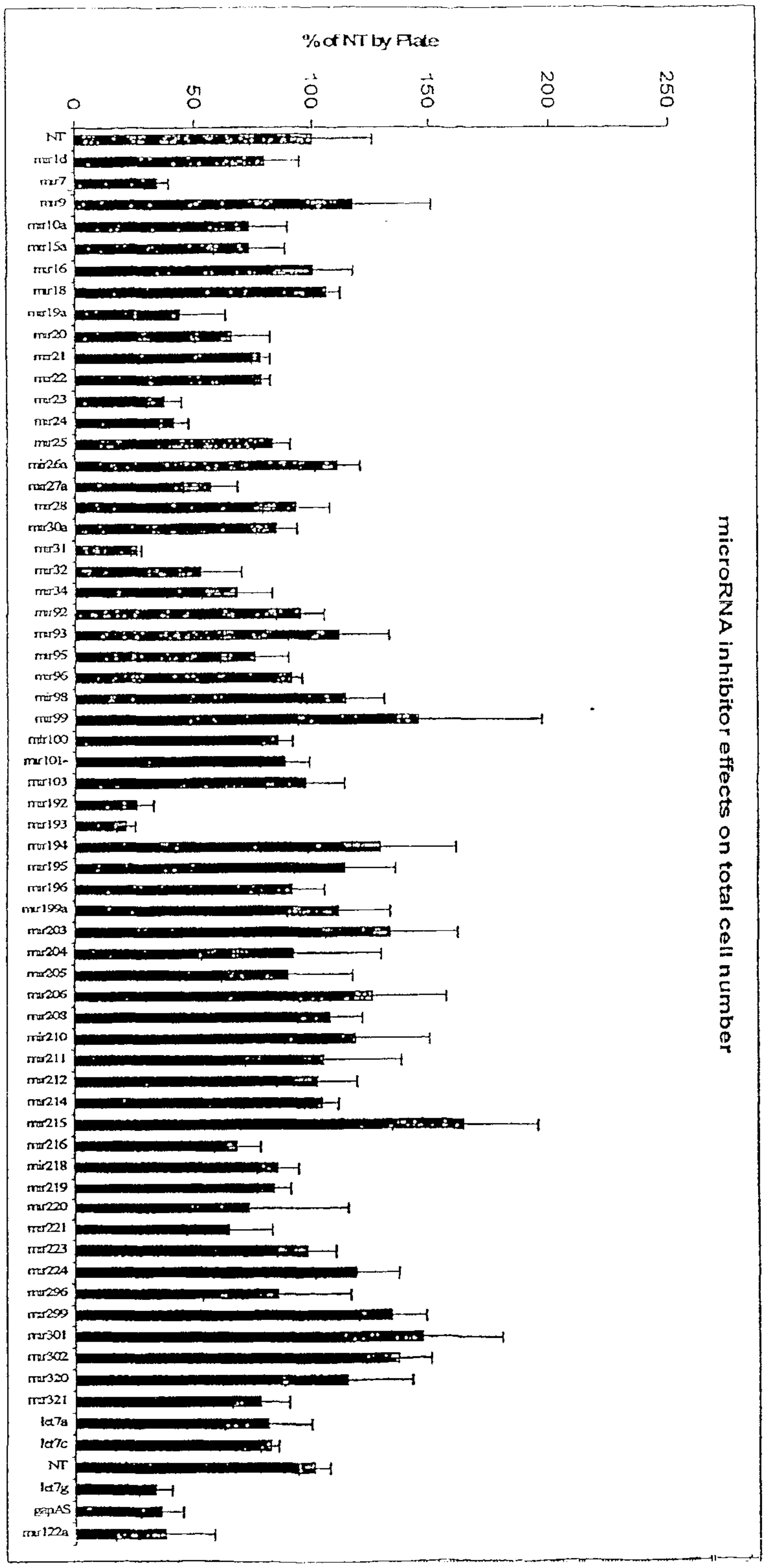

0
20
40
60
80
100
120
140
160
180
200
Reduced miR-31
Negative Control

Fig. 7



microRNA inhibitor effects on total cell number
% of NT by Plate
0
50
100
150
200
250
NT
mir1d
mir7
mir9
mir10a
mir15a
mir16
mir18
mir19a
mir20
mir21
mir22
mir23
mir24
mir25
mir26a
mir27a
mir28
mir30a
mir31
mir32
mir34
mir92
mir93
mir95
mir96
mir98
mir99
mir100
mir101-
mir103
mir192
mir193
mir194
mir195
mir196
mir199a
mir203
mir204
mir205
mir206
mir208
mir210
mir211
mir212
mir214
mir215
mir216
mir218
mir219
mir220
mir221
mir223
mir224
mir296
mir299
mir301
mir302
mir320
mir321
let7a
let7c
NT
let7g
gapAS
mir122a

Fig. 8

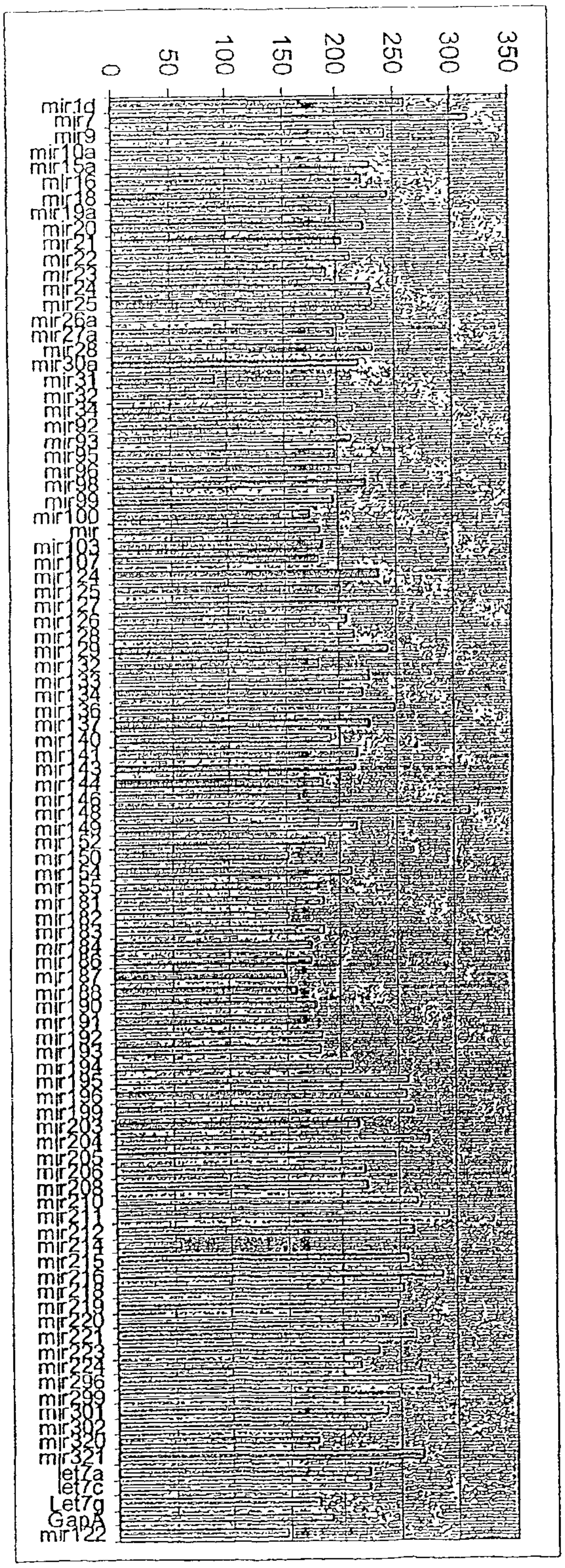

0
50
100
150
200
250
300
350
mir1d
mir7
mir9
mir10a
mir15a
mir16
mir18
mir19a
mir20
mir21
mir22
mir23
mir24
mir25
mir26a
mir27a
mir28
mir30a
mir31
mir32
mir34
mir92
mir93
mir95
mir96
mir98
mir99
mir100
mir
mir103
mir107
mir124
mir125
mir127
mir126
mir128
mir129
mir132
mir133
mir134
mir136
mir137
mir140
mir141
mir143
mir142
mir144
mir146
mir148
mir149
mir152
mir150
mir154
mir155
mir181
mir182
mir183
mir184
mir186
mir187
mir188
mir190
mir191
mir192
mir193
mir194
mir195
mir196
mir199
mir203
mir204
mir205
mir206
mir208
mir210
mir211
mir212
mir214
mir215
mir216
mir218
mir219
mir220
mir221
mir223
mir224
mir296
mir299
mir301
mir302
mir320
mir321
let7a
let7c
Let7g
GapA
mir122

Fig. 9

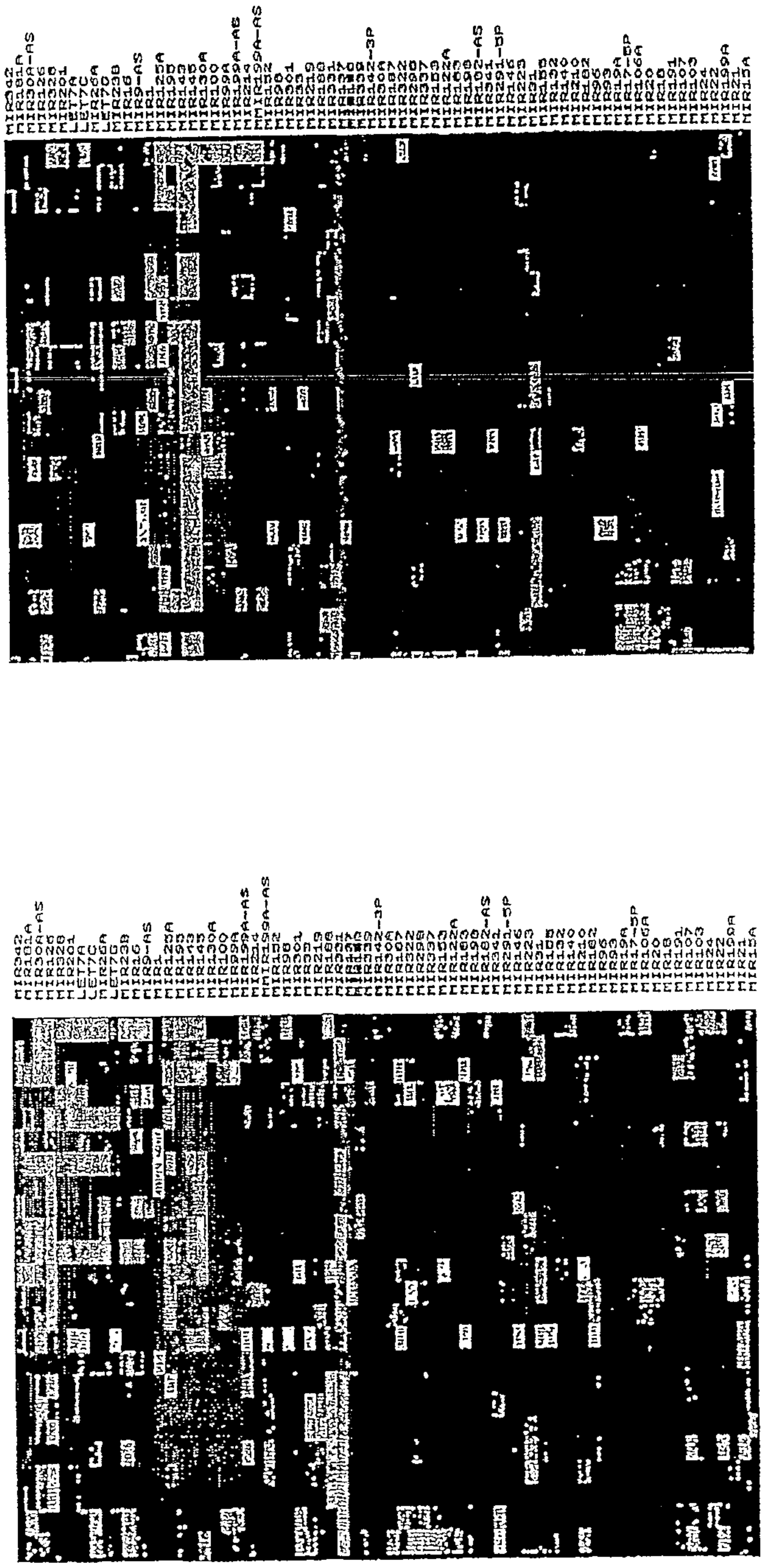


FIG. 10

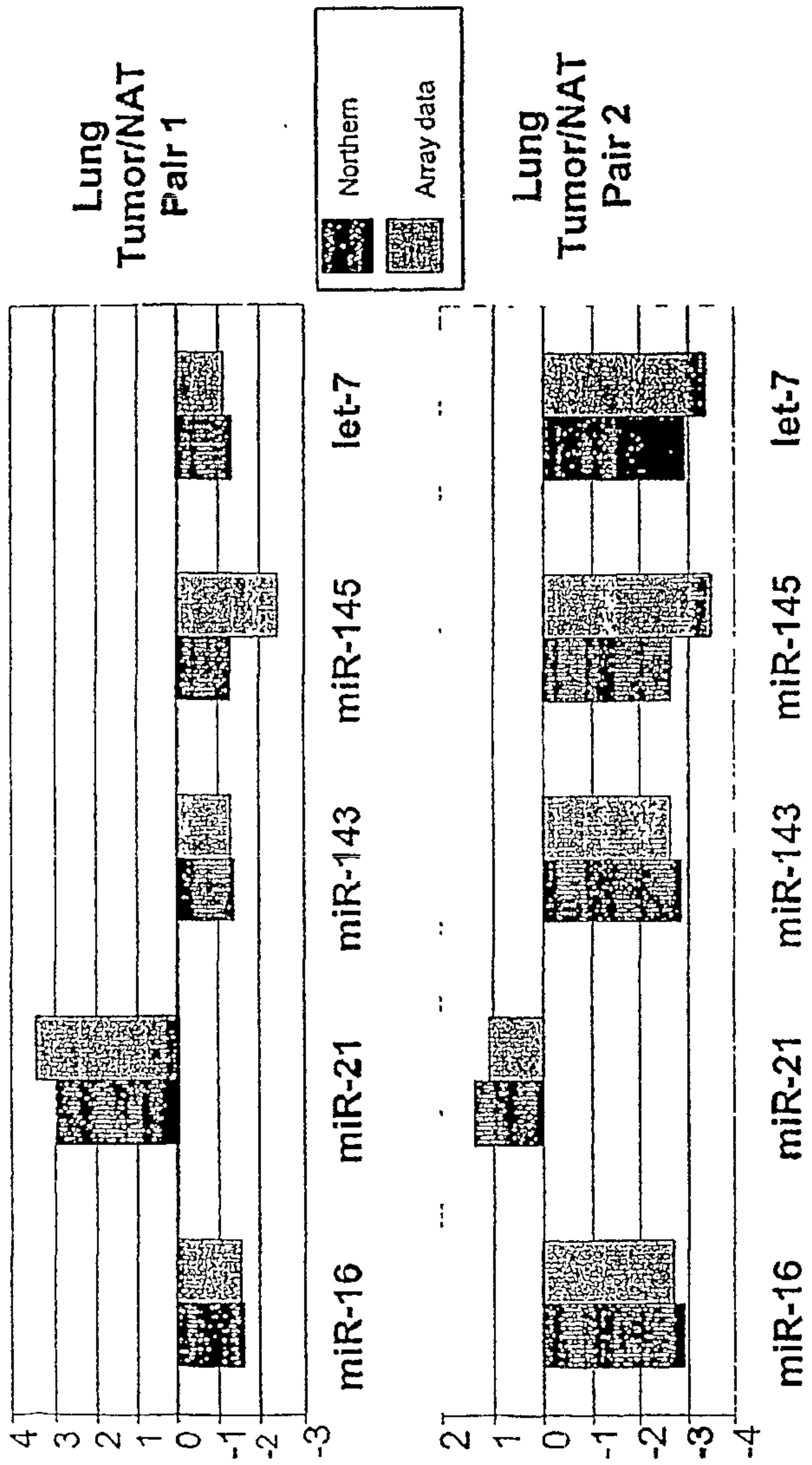

Lung Tumor/NAT Pair 1
Northern
Array data
Lung Tumor/NAT Pair 2
4
3
2
1
0
-1
-2
-3
2
1
0
-1
-2
-3
-4
miR-16
miR-21
miR-143
miR-145
let-7

FIG. 11

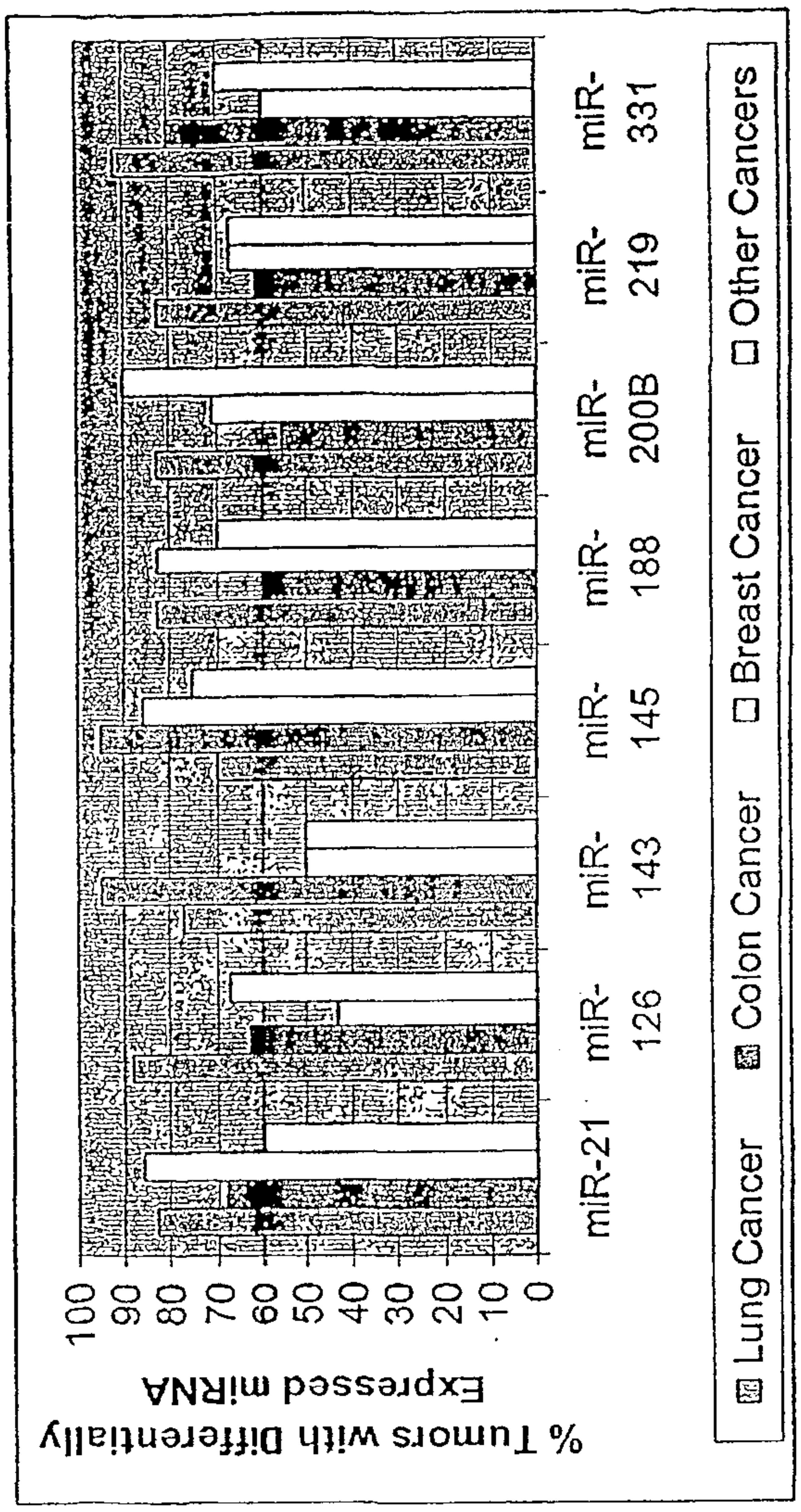

% Tumors with Differentially Expressed miRNA
100
90
80
70
60
50
40
30
20
10
0
miR-21
miR-126
miR-143
miR-145
miR-188
miR-200B
miR-219
miR-331
Lung Cancer
Colon Cancer
Breast Cancer
Other Cancers

FIG. 12

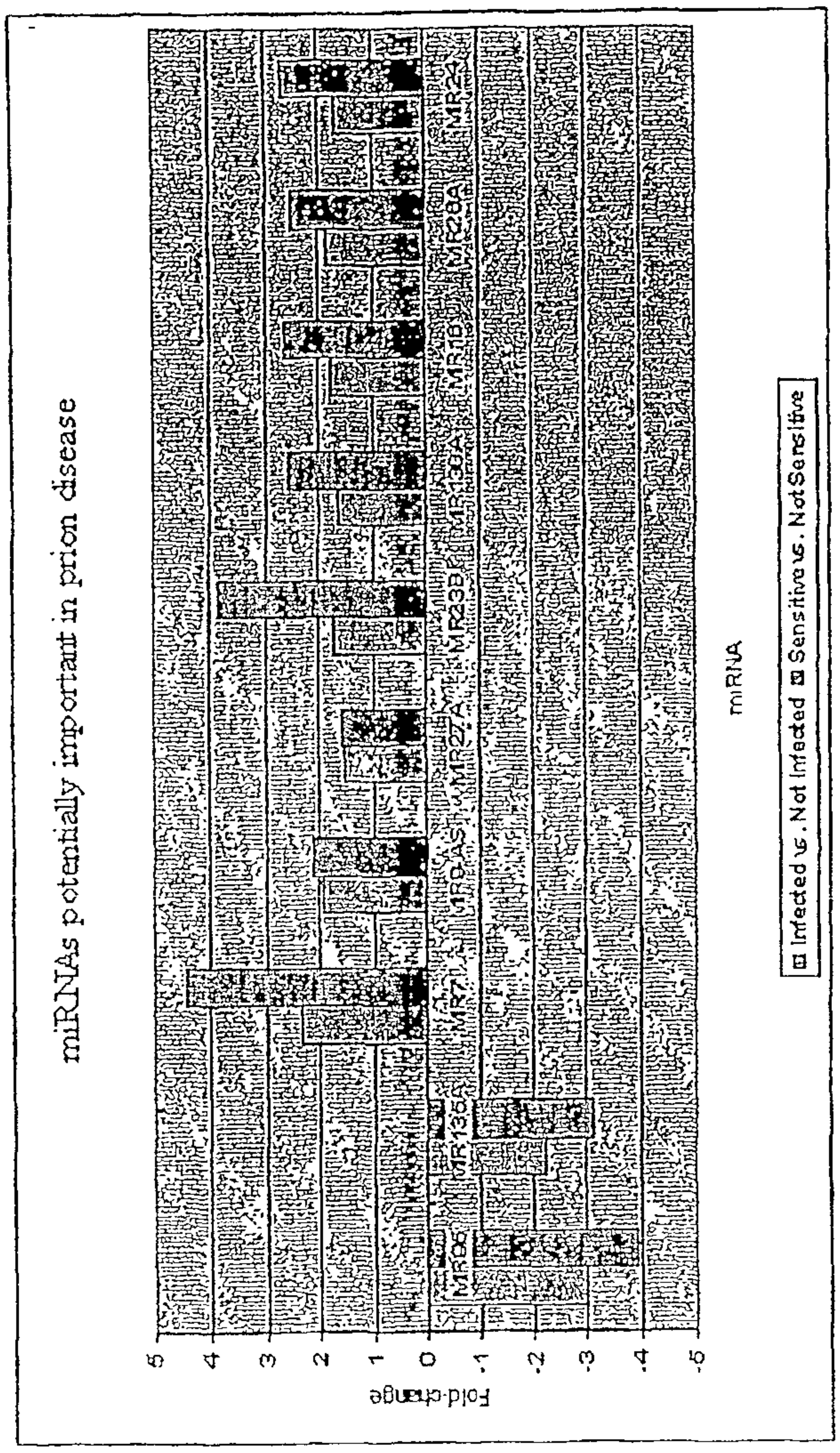

miRNAs potentially important in prion disease
Fold-change
miRNA
Infected vs. Not Infected
Sensitive vs. Not Sensitive

FIG. 13

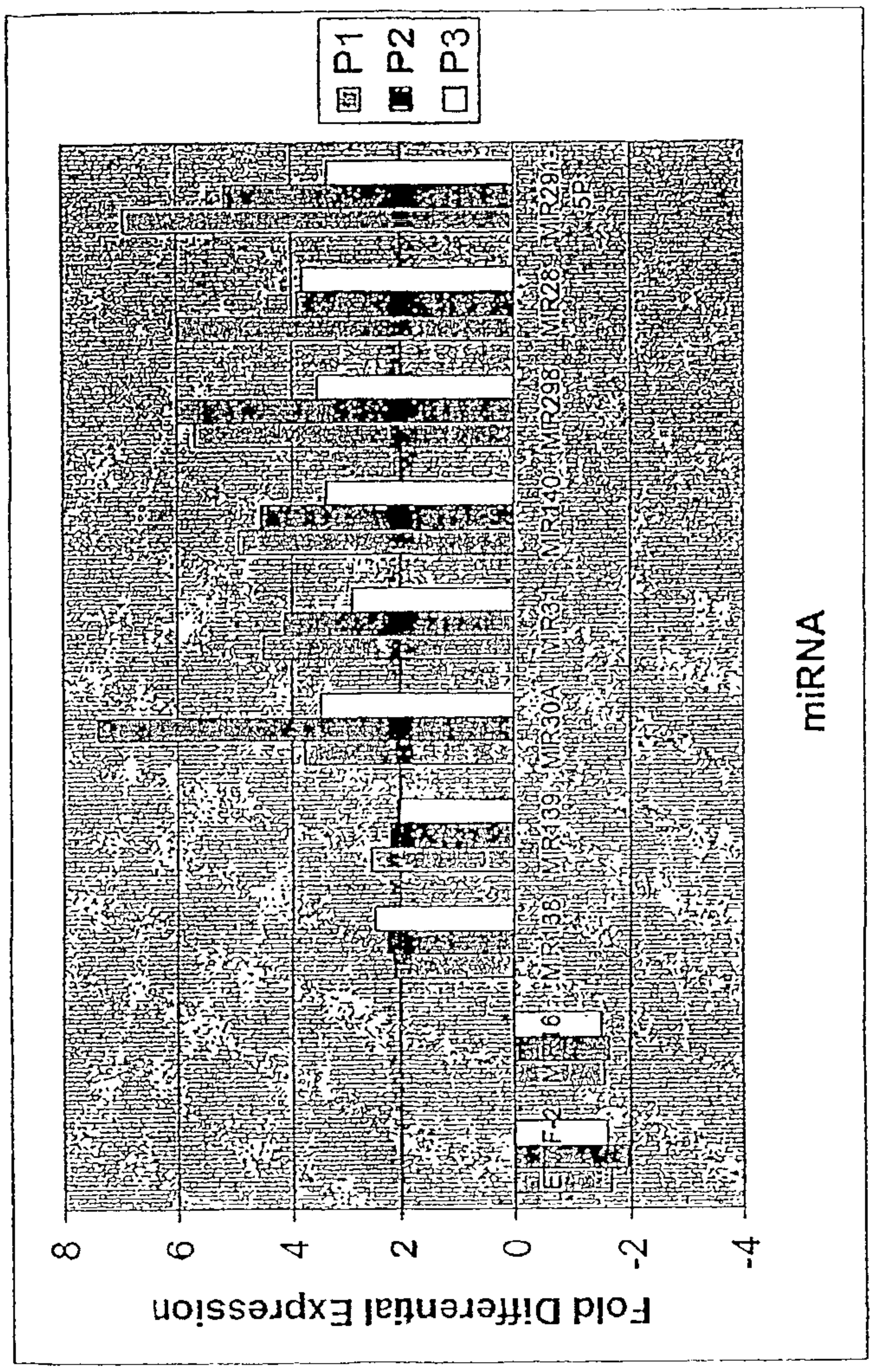


FIG. 14

Breast BT549

miRNA	% of neg cont	St Dev
mir-101	87	3
mir-105	88	2
mir-124	88	5
mir-126	82	2
mir-129	85	1
mir-132	87	4
mir-142	87	4
mir-192	81	3
mir-201	79	14
mir-215	82	1
mir-27a	87	4
mir-346	88	1
mir-92	85	11
mir-96	87	9
mir-98	87	1
mir-99a	88	1

Breast MCF12A

miRNA	% of neg cont	St Dev
mir-126	88	32
mir-142	87	27
mir-147	87	33
mir-206	86	12
mir-208	87	12
mir-210	86	11
mir-211	83	7
mir-214	85	19
mir-215	70	10
mir-219	88	10
mir-220	86	7
mir-221	88	7
mir-223	87	10
mir-331	88	12
mir-345	88	10
mir-346	82	12
mir-297	78	15
mir-329	82	17
mir-409	86	11
mir-411	86	7

Cervical HeLa

miRNA	% of neg cont	St Dev
mir-1	20	2
mir-101	12	2
mir-124	16	4
mir-192	13	3
mir-193	5	2
mir-195	21	7
mir-201	26	7
mir-206	12	6
mir-208	21	12
mir-210	23	4
mir-215	33	21
mir-299	20	18
mir-337	18	3
mir-339	31	2
mir-340	31	7
mir-345	31	21
mir-34a	35	7
mir-367	31	12
mir-292-3p	29	21
mir-293	26	30
mir-297	19	16
mir-344	32	1
mir-409	30	16

Prostate 22 Rv1

miRNA	% of neg cont	St Dev
mir-126	63	3
mir-101	77	9
mir-103	75	10
mir-105	75	12
mir-107	84	22
mir-124	77	6
mir-128	81	4
mir-129	81	4
mir-132	84	10
mir-135	79	4
mir-137	81	4
mir-141	85	2
mir-142	69	4
mir-147	66	4
mir-15a	85	9
mir-16	74	6
mir-27a	83	7
mir-28	82	9
mir-30a-3p	80	8
mir-34a	72	8
mir-297	84	5

FIG. 15A

Skin TE354T

miRNA	% of neg cont	St Dev
mir-1	53	7
mir-101	49	6
mir-124	64	10
mir-136	59	4
mir-154	51	5
mir-15a	63	4
mir-16	58	5
mir-192	62	7
mir-193	69	4
mir-195	58	3
mir-201	67	8
mir-206	51	4
mir-215	59	6
mir-221	62	8
mir-26a	69	12
mir-28	60	18
mir-346	67	6
mir-34a	65	5
mir-7	63	1
mir-96	60	8
mir-329	60	4
mir-376b	65	3

Skin TE353SK

miRNA	% of neg cont	St Dev
mir-101	63	11
mir-105	42	40
mir-124	70	8
mir-126	56	8
mir-128	53	46
mir-132	66	8
mir-133A	56	49
mir-136	27	24
mir-137	55	13
mir-141	62	23
mir-142	60	19
mir-144	65	22
mir-15a	57	20
mir-16	59	9
mir-181a	65	23
mir-20	47	41
mir-206	67	15
mir-215	68	11
mir-223	56	49
mir-302	63	1
mir-330	66	59
mir-346	59	52
mir-373	66	7
mir-96	65	16
mir-291	48	17
mir-329	68	62
mir-380-3p	64	55
mir-411	54	47

BJ cells

miRNA	% of neg cont	St Dev
miR-206	19	7
let7a	69	10
mir1	38	2.65
miR-105	65	5
miR-147	35	7
miR-15a	43	8
miR-16	51	4
miR-195	48	10
miR-297	46	6.85
miR-324-3p	68	8.84
miR-337	27	7.26
miR-376b	63	6.53

Lung A549

miRNA	% of neg cont	St Dev
mir-124	44	0
Let-7b	70	0
Let-7d	71	0
Let-7g	62	1
mir-126	68	0
mir-129	67	1
mir-137	64	0
mir-147	65	0
mir-15a	61	0
mir-16	53	1
mir-192	70	2
mir-193	64	3
mir-195	59	0
mir-22	68	1
mir-28	61	0
mir-292-3p	62	0
mir-29a	67	1
mir-337	55	0
mir-344	63	0
mir-345	69	1
mir-34a	61	0
mir-7	63	1

FIG. 15B

Lung CRL5826

miRNA	% of NC	STDEV
ambi-mir7100	63	21
mir-101	70	20
mir-105	74	11
mir-124	63	8
mir-125b	74	11
mir-126	61	3
mir-128	71	13
mir-132	73	18
mir-141	74	5
mir-142	67	5
mir-147	75	7
mir-149	71	9
mir-188	67	11
mir-223	68	14
mir-28	74	19
mir-29a	74	14
mir-337	74	17
mir-346	72	13
mir-96	74	6

Lung HTB-57

miRNA	% of NC	STDEV
mir-108	79	
mir-122	79	
mir-124	79	
mir-125a	77	11
mir-126	78	2
mir-132	59	7
mir-133A	77	7
mir-136	78	13
mir-147	72	4
mir-151	67	10
mir-152	73	13
mir-16	79	6
mir-182	63	9
mir-183	72	9
mir-186	69	17
mir-188	67	20
mir-28	79	16
mir-377	79	3
mir-526b*	79	0
mir-96	76	10

Jurkats

miRNA	% of neg cont	St Dev
let-7a	21	1
let-7b	50	5
miR-101	69	30
miR-10b	37	3
miR-122	67	18
miR-133a	73	18
miR-17-3p	63	16
miR-29a	68	7
miR-30a-3p	66	27
miR-34a	67	21

Primary T-cells

miRNA	% of neg cont	St Dev
miR-107	89	15
miR-134	75	23
miR-135	88	13
miR-139	87	0
miR-141	89	1
miR-145	86	12

FIG. 15C

Cervical HeLa

miRNA	% of neg cont	St Dev
Let-7a	119	15
Let-7b	124	12
Let-7c	114	21
Let-7d	113	29
Let-7g	114	27
mir-145	111	29
mir-155	114	17
mir-181a	113	21
mir-186	111	29
mir-190	114	21
mir-191	116	22
mir-199	118	14
mir-9	112	27

Prostate 22 Rv1

miRNA	% of neg cont	St Dev
Let-7a	124	6
Let-7b	127	27
mir-127	127	11
mir-154	123	10
mir-181a	124	11
mir-194	132	16
mir-198	126	10
mir-199	146	18
mir-201	125	24
mir-369	130	9
mir-93	129	16

Skin TE354T

miRNA	% of neg cont	St Dev
mir-139	117	20
mir-141	120	52
mir-143	122	35
mir-145	156	71
mir-146	143	85
mir-188	117	37
mir-190	131	55
mir-198	119	3
mir-204	125	8
mir-410	133	3
mir-412	125	11

Skin TE353SK

miRNA	% of neg cont	St Dev
mir-138	126	7
mir-196	134	7
mir-197	135	8
mir-198	144	4
mir-199	135	9
mir-204	125	6
mir-216	136	13
mir-410	134	7

BJ cells

miRNA	% of neg cont	St Dev
miR-26a	130	17
miR-128	131	24
miR-223	134	14
miR-188	139	19
miR-125a	140	10
miR-201	153	18
miR-291-3p	155	30
miR-145	161	2
miR-294	165	21
miR-150	171	7
miR-322	212	19.93
miR-295	215	40.78
miR-187	246	19
miR-373	268	41.39

Lung Cancer A549

miRNA	% of neg cont	St Dev
mir-25	112	0
mir-294	112	1
mir-32	121	0
mir-92	122	0

Jurkats

miRNA	% of neg cont	St Dev
miR-100	134	15
miR-125b	132	4
miR-126	134	19
miR-129	150	10
miR-140	139	4
miR-143	162	2
miR-155	146	23
miR-15a	146	12
miR-23b	135	4
miR-25	154	18
miR-26a	170	14

Primary T-cells

miRNA	% of neg cont	St Dev
let-7a	151	17
let-7b	150	14
let-7c	159	4
let-7d	142	10
let-7g	141	7
miR-10a	130	10
miR-10b	127	20
miR-125a	131	5
miR-126	126	42
miR-15a	135	11
miR-17-3p	128	4
miR-18	138	2
miR-182	126	18
miR-19a	126	5
miR-20	130	14
miR-7	126	1

CRL5826

miRNA	% of NC	STDEV
mir-130a	126	27
mir-145	112	8
mir-30e-5p	122	11
mir-333	112	35
mir-335	114	33
mir-369	111	8
mir-350	111	5
mir-412	123	14

HTB-57

miRNA	% of NC	STDEV
mir-135	121	8
mir-216	126	7
mir-293	121	4
mir-338	122	14
mir-341	118	22

FIG.16

Prostate 22Rv1

miRNA	% NC	St Dev
mir-100	59	26
mir-130a	58	16
mir-211	54	7
mir-212	58	1
mir-213	57	11
mir-215	59	8
mir-224	49	30
mir-292	59	4
mir-320	58	6
mir-324	55	6
mir-325	59	10
mir-330	58	28
mir-338	55	10
mir-369	57	6
mir-370	54	16
mir-99a	58	15

Skin TE354T

miRNA	% NC	St Dev
mir-210	67	13
mir-216	53	1

Breast MCF12a

miRNA	% NC	St Dev
mir-216	92	9
mir-217	95	14
mir-294	93	23

Lung A549

miRNA	% NC	St Dev
mir-129	90	5
mir-326	88	2
mir-331	92	2
mir-338	91	2
mir-341	89	5
mir-370	91	0
mir-92	88	0

FIG. 17

Prostate 22Rv1

miRNA	% NC	St Dev
mir-10b	104	27
mir-152	111	20

Skin TE354T

miRNA	% NC	St Dev
Let-7a	139	9
Let-7b	148	8
Let-7g	133	8
mir-10a	135	10
mir-10b	140	10
mir-133B	135	8
mir-155	138	3
mir-15a	142	12
mir-16	134	8
mir-181a	138	6
mir-182	133	9
mir-193	134	12
mir-194	137	28
mir-196	133	6
mir-204	135	9
mir-23a	133	16
mir-24	132	11
mir-25	142	13
mir-92	132	11
mir-95	137	5

Breast MCF12a

miRNA	% NC	St Dev
let7a	180	35
let7b-1	176	36
let7c	177	21
let7d	172	37
mir-10a	178	22
mir-10b	190	18
mir-133a	182	33
mir-152	175	33
mir-153	178	27
mir-155	186	24
mir-16	174	30
mir-181a	172	26
mir-183	184	15
mir-184	177	14
mir-186	176	16
mir-191	174	11
mir-200b	179	8
mir-412	174	24
mir-9	178	24

Lung A549

miRNA	% NC	St Dev
let7a-1	116	5
mir-133a-2	126	2
mir-142	112	4
mir-187	110	6
mir-199a-1	111	4
mir-206	110	4
mir-211	110	6
mir-222	111	2
mir-223	112	2
mir-23b	118	6
mir-298	111	1
mir-328	115	1
mir-342	118	0
mir-371	122	2

FIG. 18

miRNAs that Decrease Cell Viability / **miRNAs that Increase Cell Viability**

Jurkat

Cell Viability

miRNA	% NC	%SD
miRNAs that Decrease Cell Viability		
let-7a	21	1
let-7b	50	5
miR-101	69	30
miR-108	75	18
miR-10b	37	3
miR-122	67	18
miR-133a	73	18
miR-17-3p	63	16
miR-19a	73	24
miR-29a	68	7
miR-30a-3p	66	27
miR-34a	67	21
miRNAs that Increase Cell Viability		
miR-129	150	10
miR-143	162	2
miR-155	146	23
miR-15a	146	12
miR-25	154	18
miR-26a	170	14

Primary T-cell

Cell Viability

miRNA	% NC	%SD
miRNAs that Decrease Cell Viability		
miR-107	89	15
miR-134	75	23
miR-135	88	13
miR-139	87	0
miR-141	89	1
miR-145	86	12
miRNAs that Increase Cell Viability		
let-7a	151	17
let-7b	150	14
let-7c	159	4
let-7d	142	10
let-7g	141	7
miR-10a	130	10
miR-10b	127	20
miR-125a	131	5
miR-126	126	42
miR-15a	135	11
miR-17-3p	128	4
miR-18	138	2
miR-182	126	18
miR-19a	126	5
miR-20	130	14

HeLa

Cell Viability

miRNA	% NC	%STDEV
miRNAs that Decrease Cell Viability		
mir-1	20	2
mir-101	12	2
mir-124	16	4
mir-192	13	3
mir-193	5	2
mir-195	21	7
mir-206	12	6
mir-208	21	12
mir-210	23	4
mir-297	19	16
mir-299	20	18
mir-337	18	3
miRNAs that Increase Cell Viability		
mir-128	128	18
mir-139	133	12
mir-23a	159	13
mir-23b	155	12
mir-24	132	20
mir-32	133	20
mir-331	128	13

A549

Cell Viability

miRNA	%NC	%SD
miRNAs that Decrease Cell Viability		
mir-193	80	15
mir-206	80	7
mir-210	86	5
mir-292-3p	86	3
mir-293	83	2
mir-299	84	4
mir-329	85	5
mir-337	81	3
mir-345	75	6
mir-346	78	8
mir-409	86	3

FIG. 19

Prostate 22Rv1			Skin TE354T			Jurkat			HeLa		
Synthetic miRNAs that Increase Apoptosis											
miRNA	%NC	St Dev	miRNA	%NC	St Dev	miRNA	%NC	St Dev	miRNA	%NC	St Dev
Let-7g	164	17	mir-149	179	22	let-7b	201	41	let-7b	369	89
mir-1	192	20	mir-154	252	15	let-7g	152	19	let-7g	594	260
mir-10a	205	15	mir-195	174	16	miR-1	170	11	miR-1	410	65
mir-149	169	14	mir-208	189	18	miR-10b	198	24	miR-10b	378	28
mir-184	166	19	mir-214	187	14	miR-122	154	29	miR-122	303	44
mir-186	166	23	mir-217	177	21	miR-17-3p	171	12	miR-17-3p	346	68
mir-188	197	12	mir-293	234	19	miR-19a	153	6	miR-19a	312	7
mir-192	182	26	mir-299	193	12	miR-28	154	20	miR-28	347	56
			mir-328	198	17	miR-29a	155	15	miR-29a	439	63
			mir-344	204	8	miR-32	156	30	miR-32	473	209
						miR-34a	181	39	miR-34a	361	82
									let-7b	317	63
									let-7g	607	150
									miR-1	355	47
									miR-10b	404	53
									miR-122	374	61
									miR-17-3p	443	101
									miR-19a	773	70
									miR-28	402	42
Synthetic miRNAs that Decrease Apoptosis											
mir-128	54	27	Let-7b	56	10	miR-125b	75	2	miR-32	8	7
mir-21	47	23	mir-100	55	11	miR-126	66	11	mir-105	40	9
mir-216	48	37	mir-101	44	9	miR-143	68	3	mir-108	39	12
mir-223	54	36	mir-126	38	11	miR-155	67	22	mir-126	29	4
mir-23b	46	44	mir-207	59	8	miR-23b	70	14	mir-137	13	12
mir-328	22	29	mir-25	59	9	miR-26a	68	11	mir-292-3p	31	4
mir-335	40	26	mir-28	41	7	miR-98	74	27	mir-34a	38	8
mir-340	51	11	mir-29a	39	8				mir-96	32	13
mir-367	37	34	mir-30a-3p	30	6						
mir-368	53	36									
mir-380-3p	30	42									
mir-410	50	47									
mir-341	53	0									

FIG. 20

A549 +/- TRAIL		A549 +/- etoposide		HTB-57 +/- etoposide		CRL-5826 +/- etoposide		HeLa +/- etoposide	
miRNAs that reduce cell viability in the presence of a therapeutic									
miRNA	%NC	miRNA	% NC	miRNA	% NC	miRNA	% NC	miRNA	% NC
mir-101	135	mir-28	319	mir-126	171	mir-132	142	mir-124	162
mir-124	158	mir-124	139	mir-132	201	mir-182	134	mir-126	161
mir-125a	134	mir-126	141	mir-28	176	mir-28	154	mir-132	170
mir-132	147	mir-147	120	mir-337	217	mir-292-3p	212	mir-147	171
mir-136	178	mir-216	108	mir-292-3p	268			mir-216	199
mir-155	181	mir-292-3p	489	miR-7100	227			mir-28	169
mir-182	153	mir-337	251					mir-292-3p	208
mir-186	176							mir-337	285
mir-202	152								
mir-206	138								
mir-221	143								
mir-224	129								
mir-28	136								
mir-291	145								
mir-292-3p	169								
mir-297	140								
mir-302	134								
mir-372	125								
mir-373	169								
mir-376b	145								
miRNAs that increase cell viability in the presence of a therapeutic									
mir-125b	83								
mir-152	73								
mir-16	90								
mir-194	78								
mir-197	82								
mir-214	82								
mir-24	87								
mir-30a-3p	63								
mir-331	73								

FIG. 21

Figure 22. miRNAs that Affect Cell Cycle

BJ Cells

G1			S			G2/M			>2N		
miRNA	%NC	%SD	miRNA	%NC	%SD	miRNA	%NC	%SD	miRNA	%NC	%SD
let7a	90	22	mir128	46	7	mir128	69	4	mir125a	50	22
mir1	87	12	mir142	49	41	mir146	61	9	mir128	31	29
mir20	67	15	mir146	53	27	mir147	54	12	mir142	59	36
mir206	70	13	mir186	60	8	mir15a	71	20	mir146	40	45
mir21	57	8	mir187	60	15	mir195	62	7	mir16	49	21
mir26a	82	5	mir195	54	0	mir371	56	5	mir191	56	40
mir290	85	2	mir297	54	22				mir201	59	27
mir294	84	17	mir324-3p	60	13				mir224	53	17
mir373	76	8	mir337	54	17				mir324-3p	46	33
			mir376b	57	17				mir92	41	20

mir125a	120	3	let7a	117	14	mir145	145	10	mir1	171	9
mir128	150	2	mir15a	112	10	mir187	140	3	mir21	205	22
mir142	133	10	mir16	124	17	mir20	151	7	mir337	191	27
mir146	148	5	mir191	113	21	mir21	169	4	mir345	172	10
mir147	126	6	mir20	116	13	mir223	131	11	mir373	174	18
mir195	131	2	mir224	125	6	mir26a	138	4			
mir201	122	6	mir26a	113	6	mir294	135	13			
mir297	125	8	mir290	117	8	mir373	146	4			
mir320	124	2	mir345	119	32						
mir324-3p	130	4									
mir325	128	4									
mir371	135	2									
mir376b	133	2									
mir409	129	11									

HeLa Pre-miR

G1			S			G2/M		
miRNA	%NC	%SD	miRNA	%NC	%SD	miRNA	%NC	%SD
mir-1	43.5	3.3	mir-108	76.6	1.1	mir-122	38.6	7.2
mir-192	44.1	1.4	mir-122	66.3	1.7	mir-124	58.4	10.4
mir-193	32.1	1.4	mir-129	75.3	0.6	mir-126	55.7	13.5
mir-206	27.4	2.6	mir-137	62.8	1.7	mir-129	66.0	4.9
mir-220	29.3	1.9	mir-147	76.8	3.3	mir-137	46.1	13.1
mir-329	38.0	0.5	mir-324-3p	71.7	0.4	mir-147	60.0	4.0
mir-371	47.0	3.6	mir-337	64.3	3.9	mir-16	62.5	12.2
mir-409	38.9	3.6				mir-219	64.2	1.9
mir-7d	57.0	0.5				mir-337	66.7	1.0

mir-108	143.9	0.7	mir-197	163.8	1.7	mir-1	161.2	1.9
mir-122	171.7	0.3	mir-205	183.7	0.9	mir-192	171.9	4.5
mir-124	150.0	0.2	mir-220	170.9	0.7	mir-193	185.3	5.9
mir-126	153.6	0.3	mir-290	167.2	1.4	mir-206	187.9	7.1
mir-129	145.1	0.3	mir-291	200.5	0.9	mir-215	147.5	2.7
mir-137	165.9	0.2	mir-294	164.6	1.1	mir-220	170.1	3.9
mir-147	150.1	0.4	mir-295	188.2	0.9	mir-329	171.5	1.2
mir-337	147.2	0.4	mir-302	214.6	1.7	mir-371	171.6	3.0
			mir-372	203.3	0.8	mir-409	169.7	7.6
			mir-411	168.8	0.9	mir-7a	154.6	4.7
						mir-7d	159.1	2.8
						mir-7g	160.8	3.0

miRNA	sequence	A549 Proliferation (% NC)	Jurkats Proliferation (% NC)
let-7a	ugagguaguagguuguauaguu	119	21
let-7b	ugagguaguagguugugugguu	124	50
let-7c	ugagguaguagguuguaugguu	114	85
let-7d	agagguaguagguugcauagu	113	97
let-7g	ugagguaguaguuuguacagu	114	105

FIG. 23

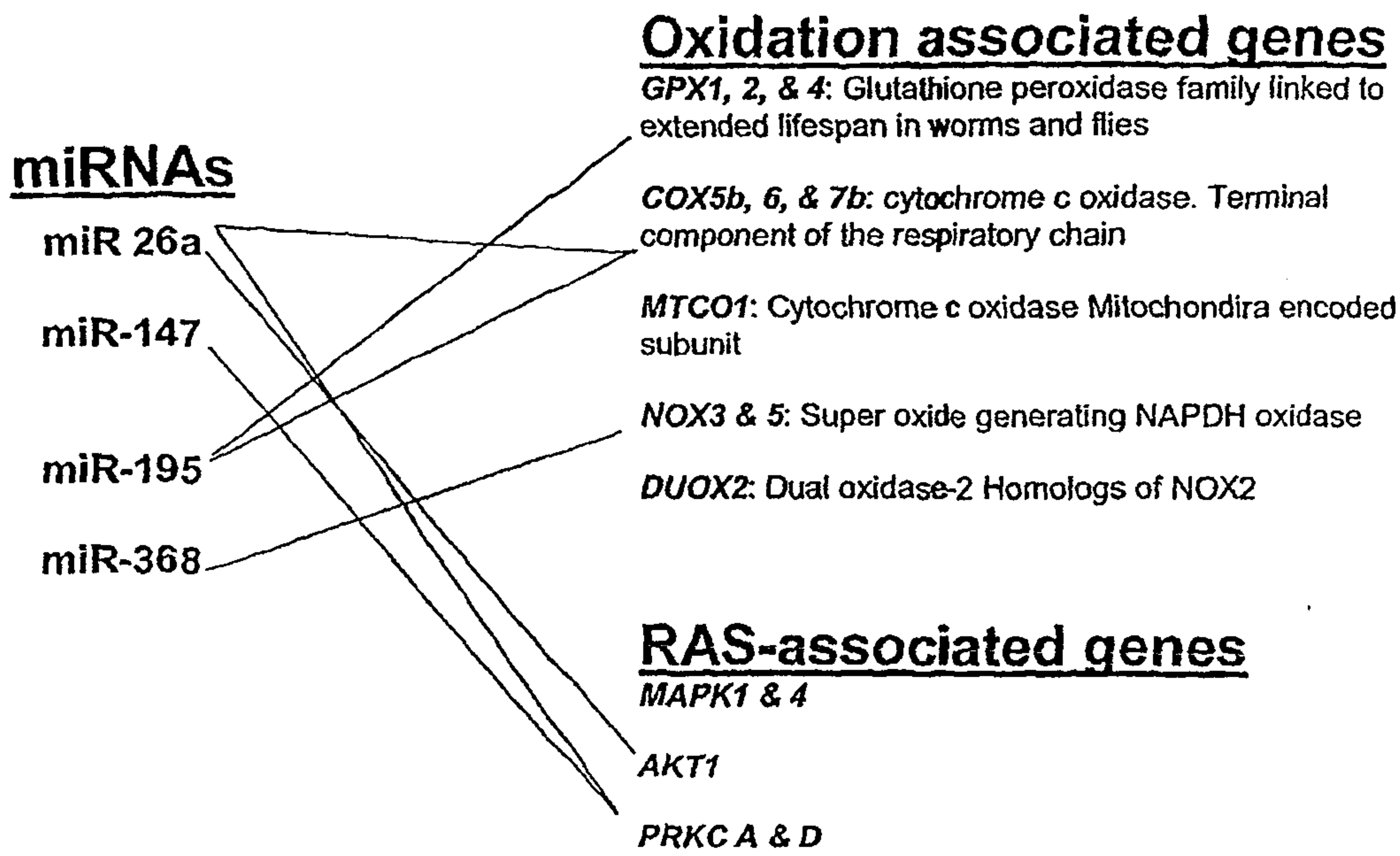

miRNAs
miR 26a
miR-147
miR-195
miR-368
Oxidation associated genes
GPX1, 2, & 4: Glutathione peroxidase family linked to extended lifespan in worms and flies
COX5b, 6, & 7b: cytochrome c oxidase. Terminal component of the respiratory chain
MTCO1: Cytochrome c oxidase Mitochondira encoded subunit
NOX3 & 5: Super oxide generating NAPDH oxidase
DUOX2: Dual oxidase-2 Homologs of NOX2
RAS-associated genes
MAPK1 & 4
AKT1
PRKC A & D

FIG. 24