

miRNA Assay	Mature (Stem-loop RT)			Mature (Linear RT)		
miRNA Target	Precursor (Ct)	Mature (Ct)	Δ Ct	Precursor (Ct)	Mature (Ct)	Δ Ct
miR-21	28.0	18.9	9.1	28.5	27.6	0.9
miR-7-1	30.2	16.1	14.1	32.9	22.5	10.4
miR-7-2	26.0		9.9	26.7		4.2
miR-218-1	28.7	15.8	12.9	24.1	16.6	7.6
miR-218-2	25.0		9.2	25.8		9.2
let-7f-1	21.6	18.2	3.4	20.4	20.8	-0.4
let-7f-2	26.8		8.6	26.9		6.1
let-7g	26.8	18.6	8.2	27.1	21.7	5.4
let-7i	21.7	18.6	3.1	20.9	20.9	0.0

TABLE 1

miRNAs	Primer Sequence	
	Cloning (Forward)	Cloning (Reverse)
miR-7-1	TAATACGACTCACTATAGGGTTGGATGTTG	CTGTAGAGGCATGGCCTGTGC
miR-7-2	TAATACGACTCACTATAGGGCTGGATACAG	TGCGATGGCTGGCACCATTAG
miR-218-1	TAATACGACTCACTATAGGGGTGATAATGTA	TGTAGAAAGCTGCGTGACGTTCC
miR-218-2	TAATACGACTCACTATAGGGGACCAAGTCGC	TGCAGGAGAGCACGGTGCTTTCCG
miR-21	TAATACGACTCACTATAGGTGTCGGGTAGC	TGTCAGACAGCCCATCGACT
let-7f-1	TAATACGACTCACTATAGGGTCAGAGTGAG	TCAGGGAAGGCAATAGATTGTATAGTTATCTCC
let-7f-2	TAATACGACTCACTATAGGGTGTGGGATGA	CGTGGGAAAGACAGTAGACTGTATAGTTATC
let-7g	TAATACGACTCACTATAGGGAGGCTGAGGT	TGGCAAGGCAGTGGCCTGTACAGTT
let-7i	TAATACGACTCACTATAGGGCTGGCTGAGG	TAGCAAGGCAGTAGCTTGCGCAGTTATCTC

TABLE 2

miRNAs	RT	Primer Sequence	
		PCR Forward	PCR Reverse
let-7a	GCTCAGACAGAAGTCACACTGAGCAACTAT	GGGCGGTGAGGTAGTAGG	GAAGTCACACTGAGCAACTATACAAC
let-7b	GCTCAGACAGAAGTCACACTGAGCAACCAC	Same to let-7a	CACACTGAGCAACCACACAAC
let-7c	GCTCAGACAGAAGTCACACTGAGCAACCAT	Same to let-7a	TCACACTGAGCAACCATACAAC
let-7d	GCTCAGACAGAAGTCACACTGAGCACTATG	GCGGGCGGAGAGGTAGT	GTCACACTGAGCACTATGCAAC
let-7e	GCTCAGACAGAAGTCACACTGAGCACTATA	GCGGGCGGTGAGGTAGG	AGAAGTCACACTGAGCACTATACAAC
let-7f	Same to let-7a	GCGGGCGGTGAGGTAGTAGA	AGAAGTCACACTGAGCACTATACAAT
let-7g	GCTCAGACAGAAGTCACACTGAGCACTGTA	GCGGGCGGTGAGGTAGTAGT	AAGTCACACTGAGCACTGTACAAA
let-7i	GCTCAGACAGAAGTCACACTGAGCACAGCA	GCGGGCGGTGAGGTAGTAGT	CACACTGAGCACAGCACAAA
miR-24	CACCGTTCCCGCGCGTGGTGCTGTTTC	CCGCGCTGGCTCAGTTC	CCGTGGTGCTGTTCTCTG
miR-92	CACCGTTCCCGCGCGTGGTGCGAGGCC	CCGCGCTATTGCACCTGTC	GTCGGTGCGAGGCCGGG
miR-10b	GACCGTTCCCGCGCGTGGTGCCAAAA	CCGCGGTACCGTGTAGAA	CGTCGGTCCAAAAATTCG
miR-221	CACCGTTCCCGCGCGTGGTGGAACCC	GCGGGCAGCTACATTGCTCTG	CGTCGGTGGAACCCAGCA
miR-222	CACCGTTCCCGCGCGTGGTGACCCAG	GCGGGCAGCTACATCTGG	CGTCGGTGACCCAGTAGC
miR-21	CACCGTTCCCGCGCGTGGTGCAACA	CCGCGCTAGCTTATCAGACTG	GCCGTGGGTGCAACATCA
miR-496-5p	CACCGTTCCCGCGCGTGGTGCTCGGG	GCGCGTCTGTACTGAGCT	GTCGGTGCTCGGGGCAG
miR-451	CACGGAACCCCGCGCGTGAACCTCA	GCGCGAAACCGTTACCAT	GCCGACCGTGAACCTAGTAAT
miR-15b	CACCGTTCCCGCGCGTGGTGCTGAAA	CCGCGGTAGCAGCACATC	CCGTGGTGCTGTAACCATG
miR-146b-5p	CACCGTTCCCGCGCGTGGTGAGCCTA	GCGGGCGTGAGAACTGAATT	CGTCGGTGAGCCTATGGA
miR-128	CACGGAACCCCGCGCGTGAAGAG	GCGGTACAGTGAACCG	CGACCGTGAAGAGACCG
miR-181c	CACGGAACCCCGCGCGTGAACCTAC	CCGCGGAACATTCAACCT	CGACCGTGAACCTACCGAC
miR-181a	Same to miR-181c	GCGCGAACATTCAACGC	Same to miR-181c
miR-181b	CACGGAACCCCGCGCGTGAACCCAC	CCGCGGAACATTCAATGC	GACCGTGACCCACCGAC
miR-425	GACCGTTCCCGCGCGTGGTCTCAACG	GGGCGAATGACACGATCAC	CGTCGGTCTCAACGGGAG
miR-7	CACCGTTCCCGCGCGTGGTGACAACA	GCGCGTGAAGACTAGTGAT	CCGTGGTGACAACAAAAT
miR-124	CACCGTTCCCGCGCGTGGTGCGGCATT	CATACCTAAGGCACGCGG	GTCGGTGCGGCATTACCC
miR-137	CACCGTTCCCGCGCGTGGTGCTACGC	CCGCGGTATTGCTTAAGAA	CGTCGGTGCTACGCGTAT
miR-139-5p	CACCGTTCCCGCGCGTGGTGCTGGAG	CCGCGCTACAGTGCAAGT	CGTCGGTGCTGGAGACAC
miR-218	CACCGTTCCCGCGCGTGGTGACATGG	TCGGGCTTGTGCTTGATCT	CCGTGGTGACATGGTTAG

TABLE 3

miRNA	RT	Oligonucleotide Sequence	T _m (°C)	ΔG _{RT} (kcal/mol)	ΔG _{PCR} (kcal/mol)
miR-21	Stem-loop	<u>CACCGTTCCCGCGCGTGGTGCTCAACA</u>	86.2	-2.37	0.19
	Linear	<u>GACCGTTCCCGCGCGTGGTGCTCAACA</u>	85.6	-0.16	1.21
miR-7	Stem-loop	<u>CACCGTTCCCGCGCGTGGTGACACA</u>	86.2	-2.98	-0.55
	Linear	<u>GACCGTTCCCGCGCGTGGTGACACA</u>	85.6	-0.16	1.21
miR-218	Stem-loop	<u>CACCGTTCCCGCGCGTGGTGACATGG</u>	87.1	-2.98	-0.55
	Linear	<u>GACCGTTCCCGCGCGTGGTGACATGG</u>	86.4	-0.16	1.21
let-7f	Stem-loop	<u>GCTCAGACAGAAGTCACACTGAGCAACTAT</u>	71.0	-3.22	-0.20
	Linear	<u>CGAGAGTCAGAAGTCACACTGAGCAACTAT</u>	70.9	-0.02	1.40
let-7g	Stem-loop	<u>GCTCAGACAGAAGTCACACTGAGCACTGTA</u>	72.8	-3.22	-0.20
	Linear	<u>CGAGAGTCAGAAGTCACACTGAGCACTGTA</u>	72.8	-0.02	1.40
let-7i	Stem-loop	<u>GCTCAGACAGAAGTCACACTGAGCACAGCA</u>	77.2	-3.22	-0.20
	Linear	<u>CGAGAGTCAGAAGTCACACTGAGCACAGCA</u>	77.2	-0.50	1.17

TABLE 4

<u>SEQUENCE</u>	<u>SEQ ID NO</u>
TAATACGACTCACTATAGGGTTGGATGTTG	1
CTGTAGAGGCATGGCCTGTGC	2
TAATACGACTCACTATAGGGCTGGATACAG	3
TGCGATGGCTGGCACCATTAG	4
TAATACGACTCACTATAGGGGTGATAATGTA	5
TGTAGAAAGCTGCGTGACGTTCC	6
TAATACGACTCACTATAGGGGACCAGTCGC	7
TGCAGGAGAGCACGGTGCTTTCCG	8
TAATACGACTCACTATAGGTGTCGGGTAGC	9
TGTCAGACAGCCCATCGACT	10
TAATACGACTCACTATAGGGTCAGAGTGAG	11
TCAGGGAAGGCAATAGATTGTATAGTTATCTCC	12
TAATACGACTCACTATAGGGTGTGGGATGA	13
CGTGGGAAAGACAGTAGACTGTATAGTTATC	14
TAATACGACTCACTATAGGGAGGCTGAGGT	15
TGGCAAGGCAGTGGCCTGTACAGTT	16
TAATACGACTCACTATAGGGCTGGCTGAGG	17
TAGCAAGGCAGTAGCTTGCGCAGTTATCTC	18
GCTCAGACAGAAGTCACACTGAGCAACTAT	19
GGGCGGTGAGGTAGTAGG	20
GAAGTCACACTGAGCAACTATACAAC	21
GCTCAGACAGAAGTCACACTGAGCAACCAC	22
CACACTGAGCAACCACACAAC	23
GCTCAGACAGAAGTCACACTGAGCAACCAT	24
TCACACTGAGCAACCATACAAC	25
GCTCAGACAGAAGTCACACTGAGCACTATG	26
GCGGGCGGAGAGGTAGT	27
GTCACACTGAGCACTATGCAAC	28
GCTCAGACAGAAGTCACACTGAGCACTATA	29
CGGGCGGTGAGGTAGG	30
AGAAGTCACACTGAGCACTATACAAC	31
CGGGCGGTGAGGTAGTAGA	32
AGAAGTCACACTGAGCAACTATACAAT	33
GCTCAGACAGAAGTCACACTGAGCACTGTA	34

TABLE 5

<u>SEQUENCE</u>	<u>SEQ ID NO</u>
CGGGCGGTGAGGTAGTAGT	35
AAGTCACACTGAGCACTGTACAAA	36
GCTCAGACAGAAGTCACACTGAGCACAGCA	37
CGGGCGGTGAGGTAGTAGT	38
CACACTGAGCACAGCACAAA	39
CACCGTCCCCGCCGTCGGTGCTGTTT	40
CCCGCCTGGCTCAGTTC	41
CCGTCGGTGCTGTTCTG	42
CACCGTCCCCGCCGTCGGTGAGGCC	43
CCCGCCTATTGCACTTGTC	44
GTCGGTGAGGCCGGG	45
GACCGTCCCCGCCGTCGGTCCACAAA	46
CCGCCGTACCCTGTAGAA	47
CGTCGGTCCACAAATTCG	48
CACCGTCCCCGCCGTCGGTGAAACC	49
CGGGCAGCTACATTGTCTG	50
CGTCGGTGAAACCAGCA	51
CACCGTCCCCGCCGTCGGTGACCCAG	52
CGGGCAGCTACATCTGG	53
CGTCGGTGACCCAGTAGC	54
CACCGTCCCCGCCGTCGGTGCAACA	55
CCCGCCTAGCTTATCAGACTG	56
GCCGTCGGTGCAACATCA	57
CACCGTCCGCGCCGTCGGTGCTCGGG	58
CGCCGTCCTGTAAGAGCT	59
GTCGGTGCTCGGGCAG	60
CACGGAACCCGCCGACCGTGAACTCA	61
CGCCGAAACCGTTACCAT	62
GCCGACCGTGAACTCAGTAAT	63
CACCGTCCCCGCCGTCGGTGTAATA	64
CCGCCGTAGCAGCACATC	65
CCGTCGGTGTAACCATG	66
CACCGTCCCCGCCGTCGGTGAGCCTA	67
CGGGCGTGAGAACTGAATT	68

TABLE 5 (continued)

<u>SEQUENCE</u>	<u>SEQ ID NO</u>
CGTCGGTGAGCCTATGGA	69
CACGGAACCCCGCCGACCGTGAAAGAG	70
GGCGTCACAGTGAACCG	71
CGACCGTGAAAGAGACCG	72
CACGGAACCCCGCCGACCGTGACTCAC	73
CCGCCGAACATTCAACCT	74
CGACCGTGACTCACCGAC	75
CGCCGAACATTCAACGC	76
CACGGAACCCCGCCGACCGTGACCCAC	77
CCGCCGAACATTCAATGC	78
GACCGTGACCCACCGAC	79
GACCGTTCCCGCCGTCGGTCTCAACG	80
GGGCGAATGACACGATCAC	81
CGTCGGTCTCAACGGGAG	82
CACCGTTCCCGCCGTCGGTGACAACA	83
CGCCCTGGAAGACTAGTGAT	84
CCGTCGGTGACAACAAAAT	85
CACCGTTCCCGCCGTCGGTGGGCATT	86
CATACCTAAGGCACGCGG	87
GTCGGTGGGCATTACCC	88
CACCGTTCCCGCCGTCGGTGCTACGC	89
CCGCCGTTATTGCTTAAGAA	90
CGTCGGTGCTACGCGTAT	91
CACCGTTCCCGCCGTCGGTGCTGGAG	92
CCGCCTCTACAGTGACCGT	93
CGTCGGTGCTGGAGACAC	94
CACCGTTCCCGCCGTCGGTGACATGG	95
TCGGGCTTGCTTGATCT	96
CCGTCGGTGACATGGTTAG	97
CACCGTTCCCGCCGTCGGTGTCACAACA	98
GACCCCTCGCGGCCGTCGGTGTCACAACA	99
CACCGTTCCCGCCGTCGGTGACAACA	100
GACCCCTCGCGGCCGTCGGTGACAACA	101
CACCGTTCCCGCCGTCGGTGACATGG	102

TABLE 5 (continued)

<u>SEQUENCE</u>	<u>SEQ ID NO</u>
GACCCCTTCGCGGCCGTCGGTGACATGG	103
GCTCAGACAGAAGTCACACTGAGCAACTAT	104
CGAGAGTCAGAAGTCACACTGAGCAACTAT	105
GCTCAGACAGAAGTCACACTGAGCACTGTA	106
CGAGAGTCAGAAGTCACACTGAGCACTGTA	107
GCTCAGACAGAAGTCACACTGAGCACAGCA	108
CGAGAGTCAGAAGTCACACTGAGCACAGCA	109
UAGCUUAUCAGACUGAUGUUGA	110
TCAACATCAGTCTGATAAGCTA	111
TAGCTTATCAGACTGATGTTGA	112
CACCGTTCCCGCCGTCGGTGTCACAA	113
CCCGCCTAGCTTATCAGACTG	114
GCCGTCGGTGTCACATCA	115
UGAGGUAGUAGGUUGUAUAGUU	116
UGAGGUAGUAGGUUGUGUGGUU	117
UGAGGUAGUAGGUUGUAUGGUU	118
UGAGGUAGUAGAUUGUAUAGUU	119
UGAGGUAGUAGUUUGUACAGU	120
UGAGGUAGUAGUUUGUGCUGU	121
AGAGGUAGUAGGUUGCAUAGU	122
UGAGGUAGGAGGUUGUAUAGU	123
GCTCAGACAGAAGTCACACTGAGCAACTAT	124
GCTCAGACAGAAGUCACACTGAGCAACTAT	125
GCTCAGACAGAAGTCACACUGAGCAACTAT	126
CACCGTTTTCTTCGGTGTCACAA	127
CACCGUUUUCUUUCGGTGTCACAA	128

TABLE 5 (continued)

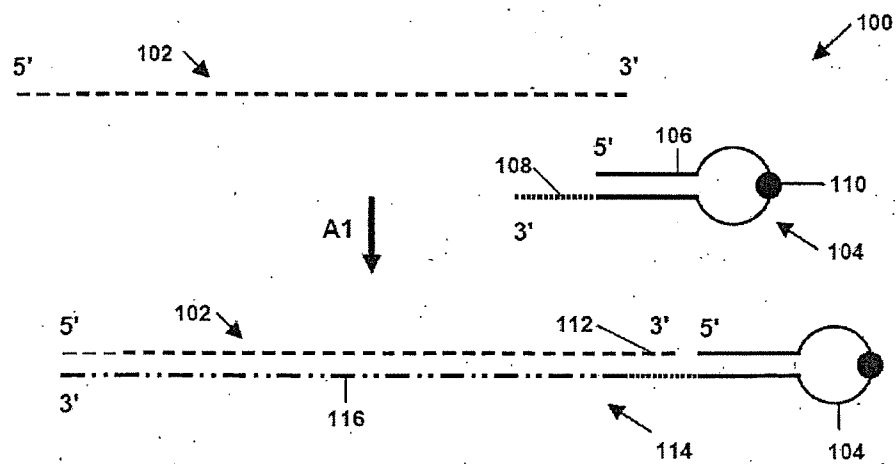


FIGURE 1

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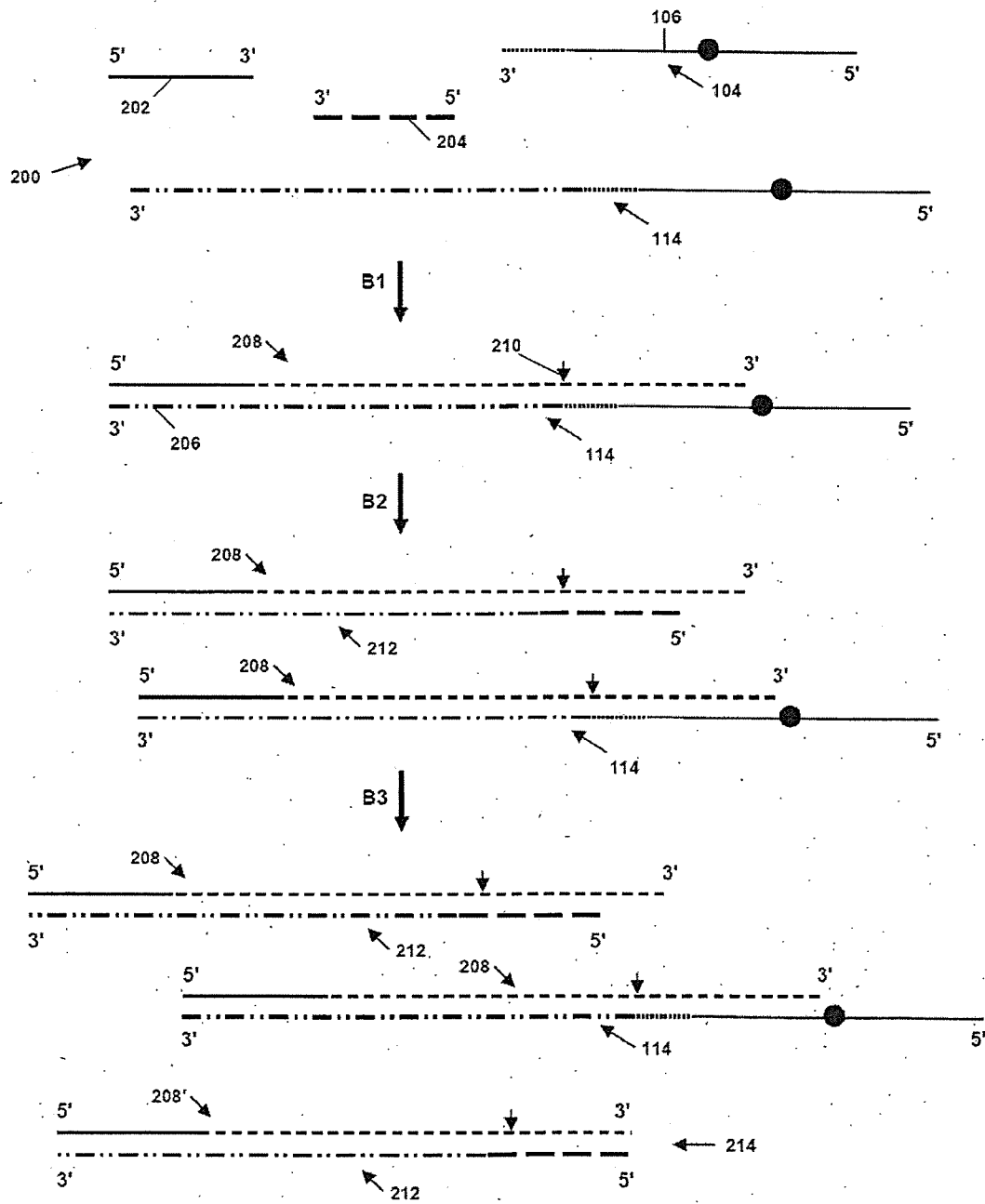


FIGURE 2

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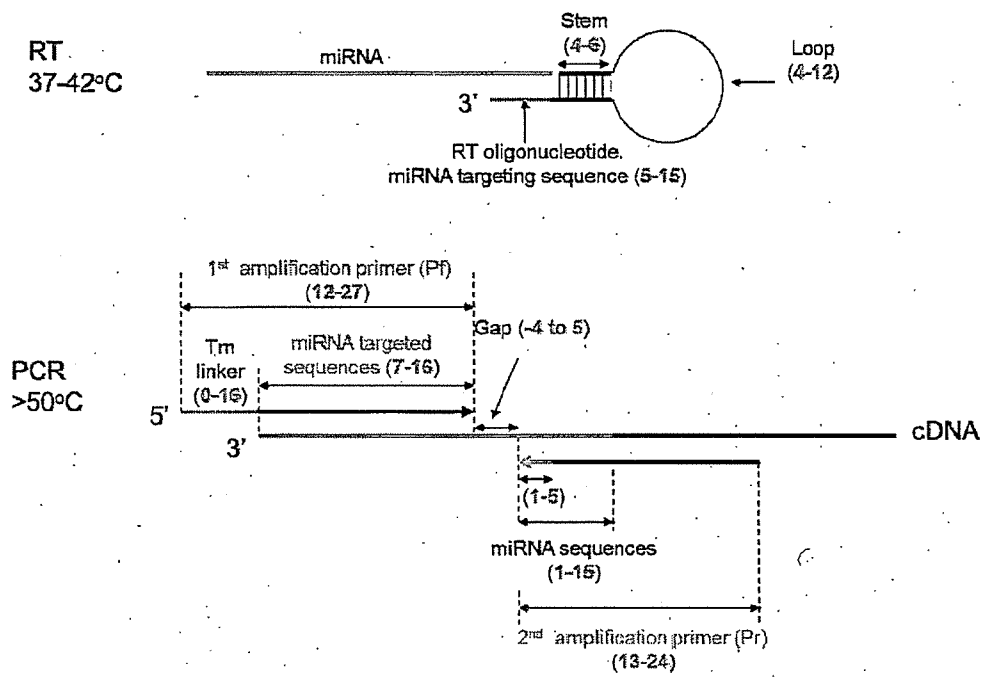


FIGURE 3

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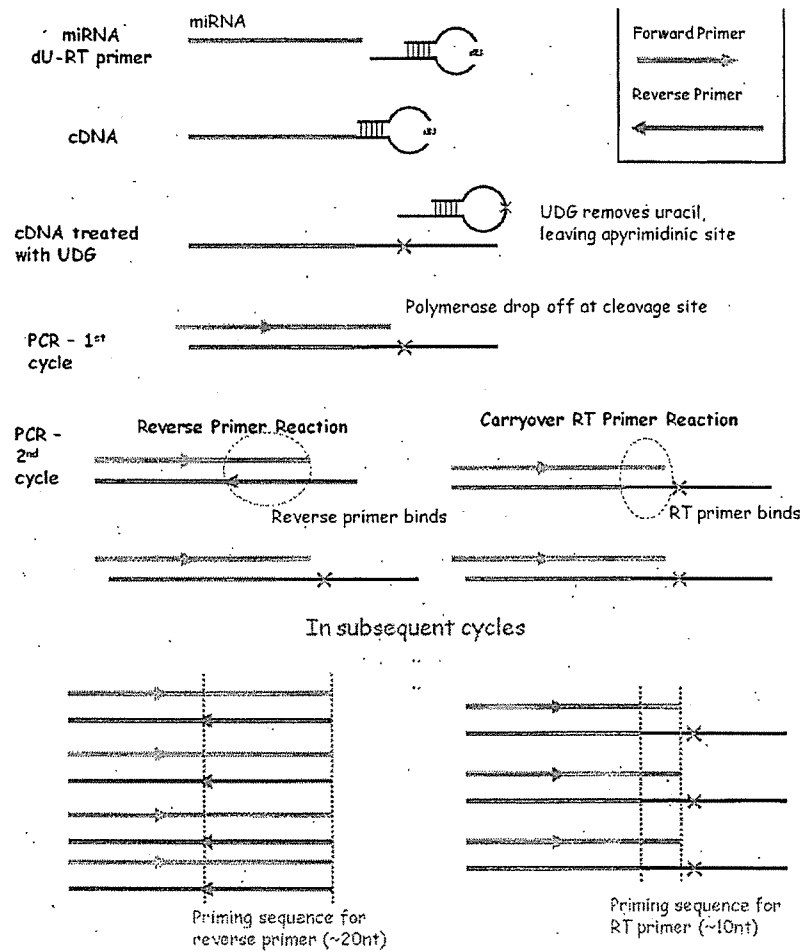
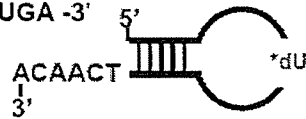


FIGURE 4

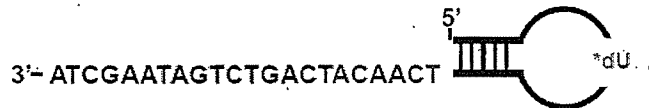
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MicroRNA target

5'- UAGCUUAUCAGACUGAUGUUGA -3'



Stem-loop Reverse Transcription



* Uracil-DNA Glycosylation

3'- ATCGAATAGTCTGACTACAACT ————— 5'



Hemi-nested Fast-cycling Real-time PCR
(SYBR Green I Detection)

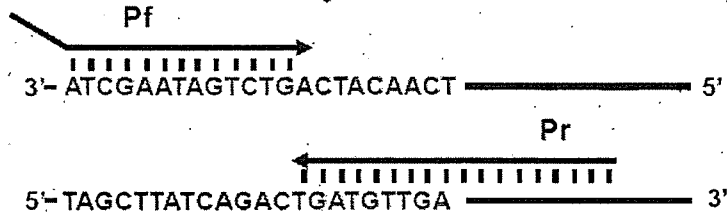
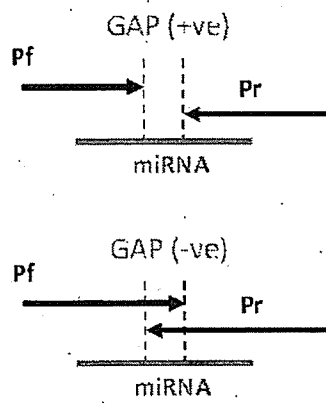


FIGURE 5

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GAP

miR-21: -2

miR-24: +2

miR-92: -1

miR-218: -2

FIGURE 6

Loop	Ct		ΔCt
	Large (11/12nt)	Small (4nt)	(Ct _{small loop} - Ct _{large loop})
miR-21	30.2	29.1	-1.0
miR-218	22.4	22.3	-0.2
miR-24	22.4	22.1	-0.3

FIGURE 7

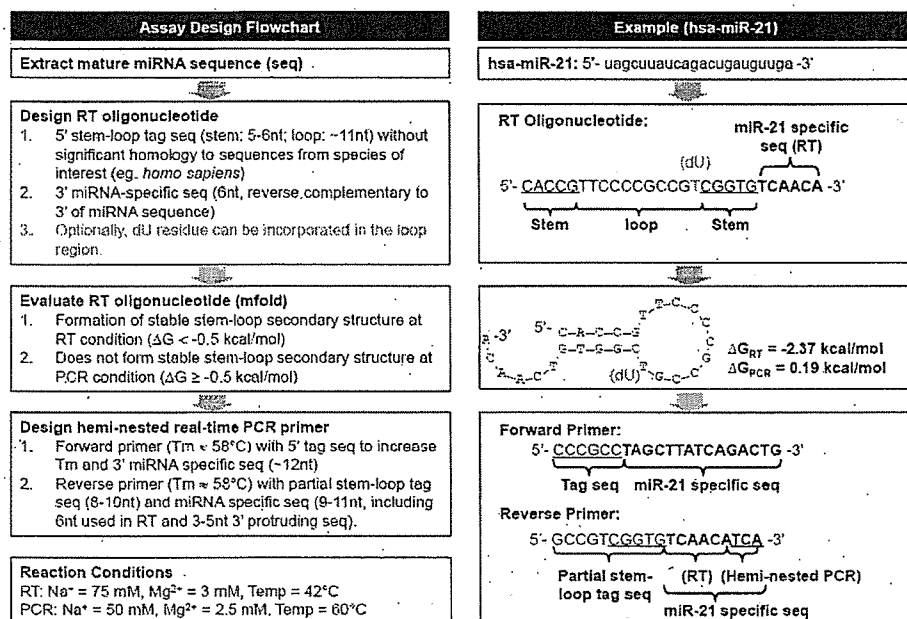


FIGURE 8

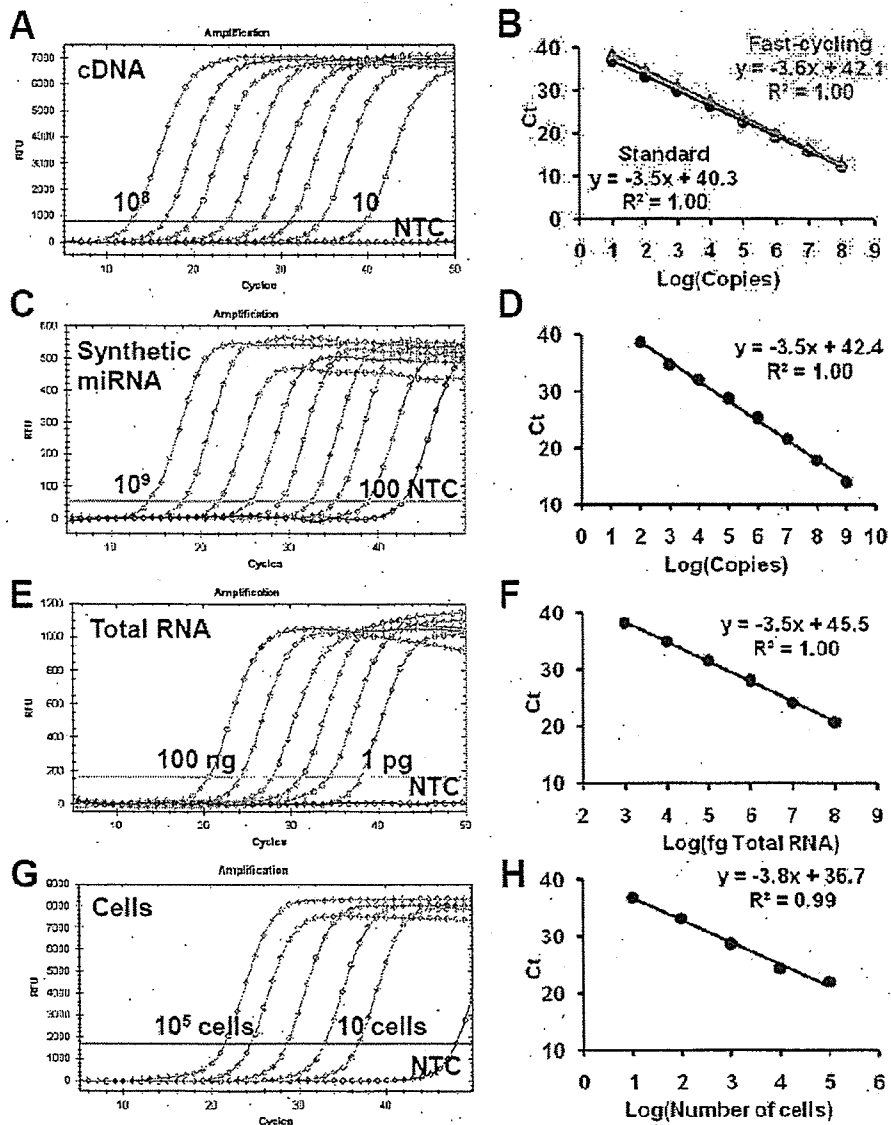


FIGURE 9

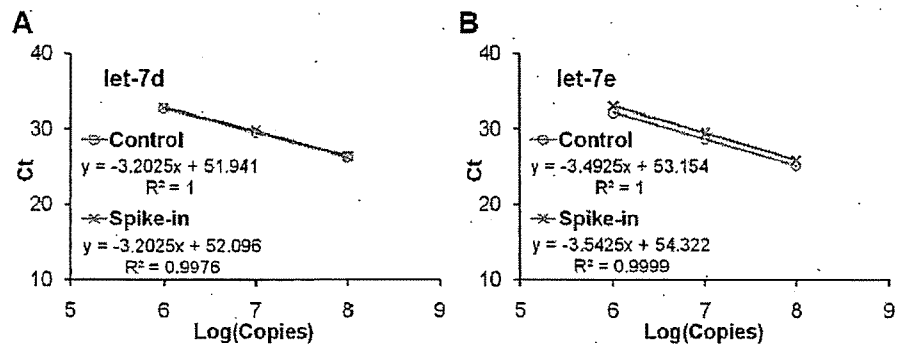


FIGURE 10

A

let-7a UCAGGUAGUACGUUGUAUAGUU
 let-7b UCAGGUAGUACGUUGUGGCUU
 let-7c UCAGGUAGUACGUUGUAUGCUU
 let-7f UCAGGUAGUACAUUGUAUAGUU
 let-7g UCAGGUAGUACUUGUAUAGUU
 let-7i UCAGGUAGUACUUGUGGCUU
 let-7d ACAGGUAGUACGUUGCAUAGUU
 let-7e UCAGGUAGUACGUUGUAUAGUU

B

Relative Detection (%)		miRNA real-time RT-PCR assay							
		let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i
Synthetic miRNA target	let-7a	100.0	0.001	0.036	0.005	0.225	0.298	0.000	0.000
	let-7b	0.002	100.0	0.248	0.002	0.001	0.001	0.000	0.000
	let-7c	1.778	0.178	100.0	0.001	0.003	0.075	0.000	0.000
	let-7d	0.072	0.000	0.000	100.0	0.000	0.000	0.000	0.000
	let-7e	0.002	0.000	0.000	0.000	100.0	0.000	0.000	0.000
	let-7f	0.029	0.000	0.000	0.002	0.002	100.0	0.000	0.000
	let-7g	0.000	0.000	0.000	0.000	0.001	0.000	100.0	0.000
	let-7i	0.000	0.000	0.000	0.001	0.001	0.000	0.001	100.0

FIGURE 11

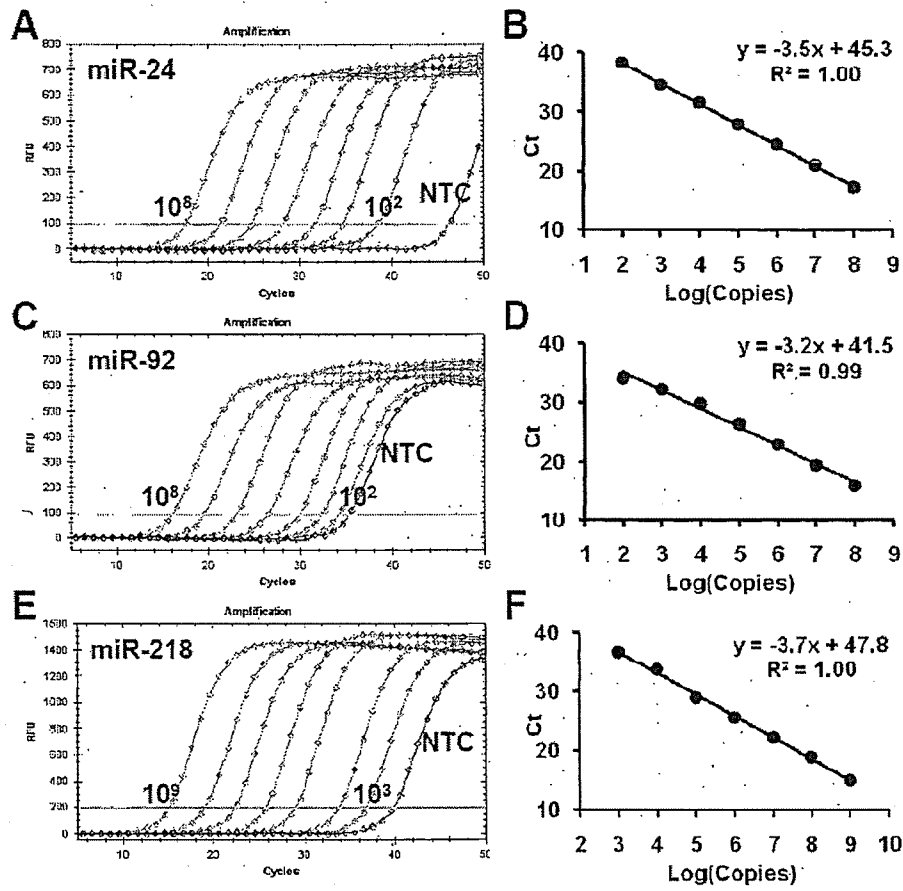
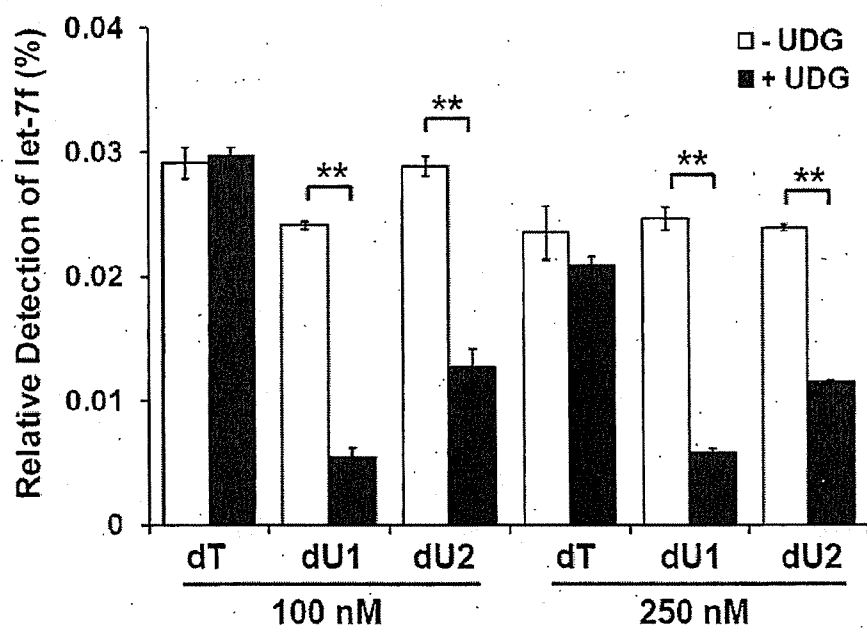


FIGURE 12

A

let-7a RT oligonucleotide	Sequence
dT	GCTCAGACAGAAGTCACACTGAGCAACTAT
dU1	GCTCAGACAGAAG <u>U</u> CACACTGAGCAACTAT
dU2	GCTCAGACAGAAGTCACAC <u>U</u> GAGCAACTAT

B**FIGURE 13**

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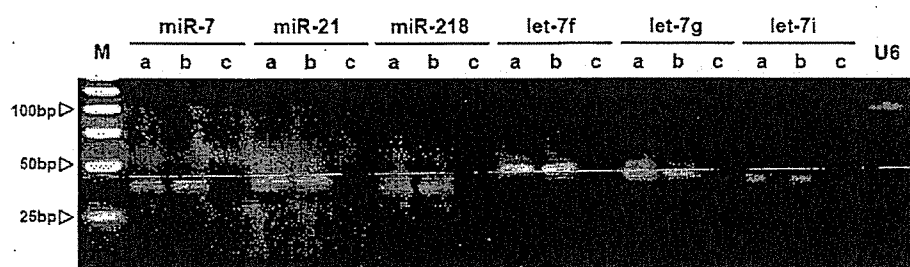


FIGURE 14

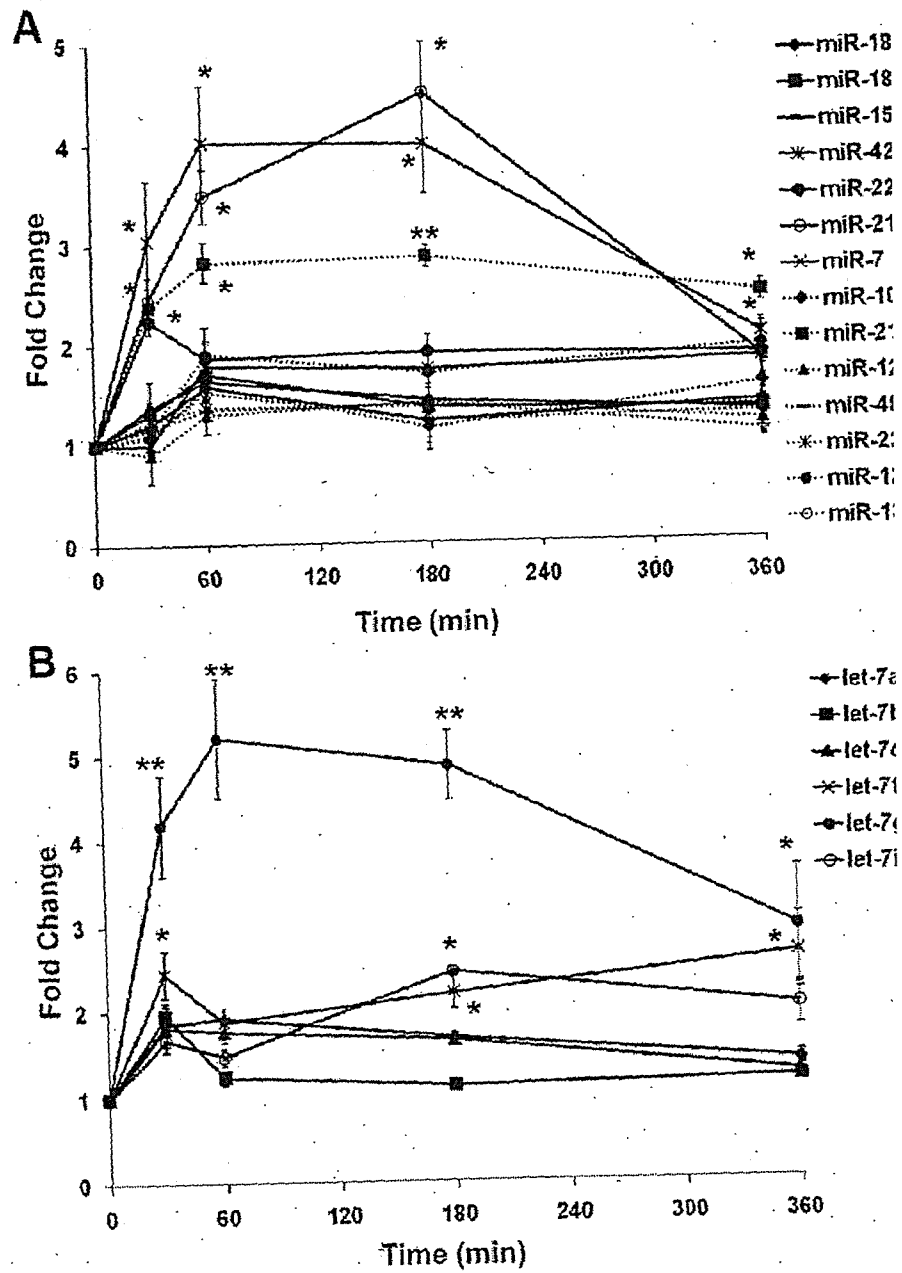


FIGURE 15

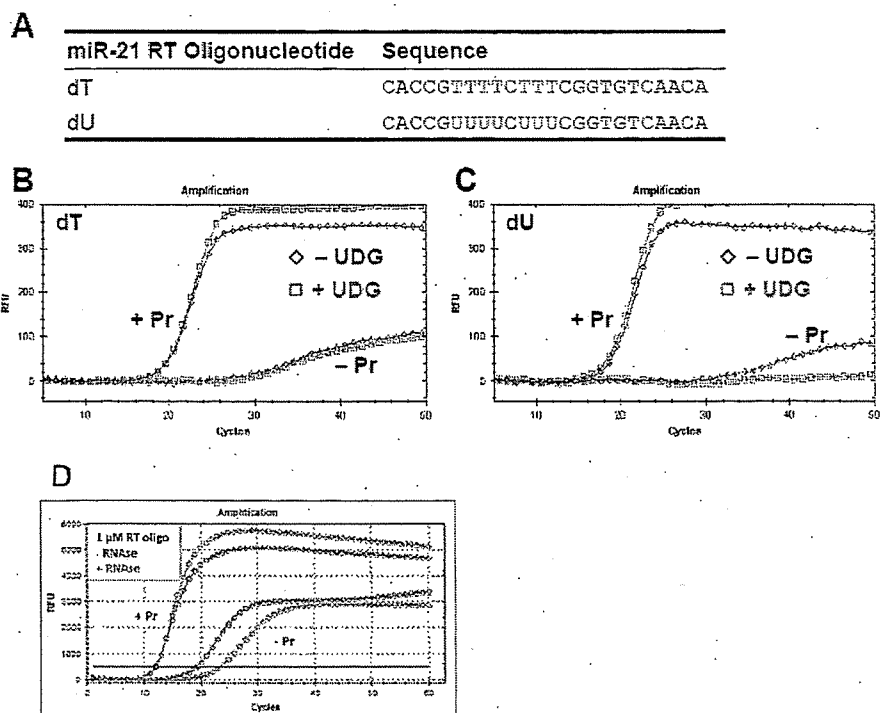


FIGURE 16

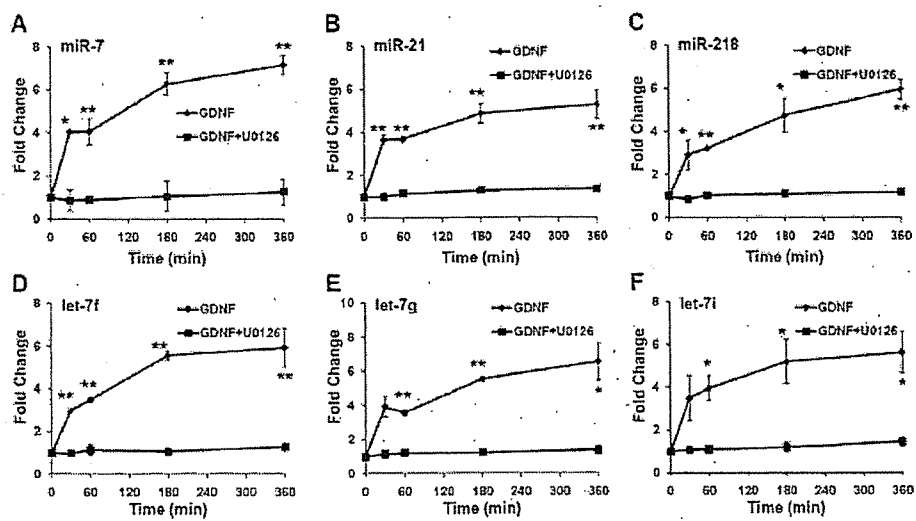


FIGURE 17

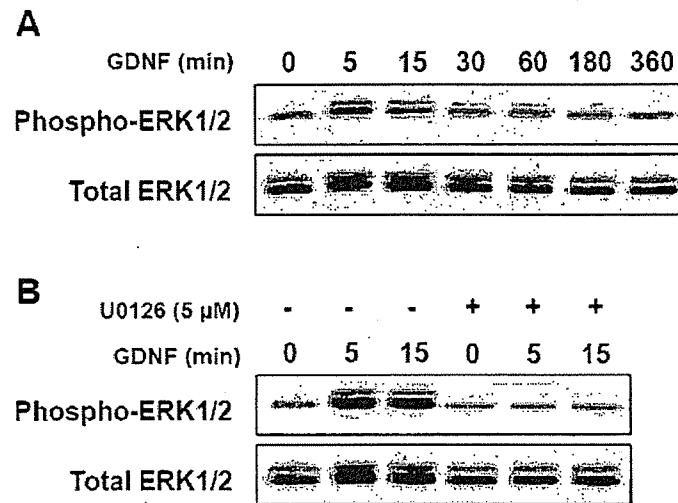


FIGURE 18

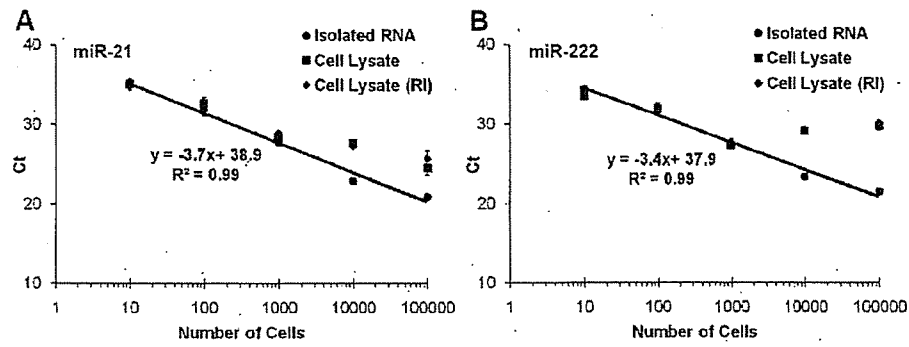


FIGURE 19

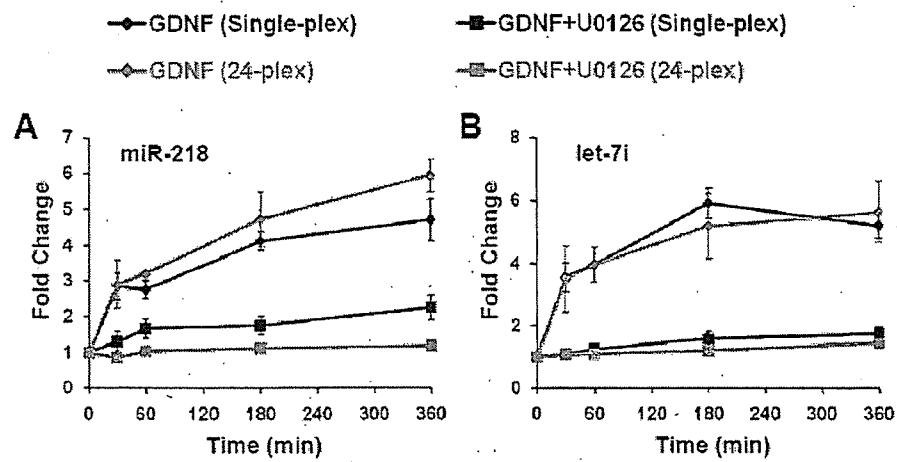


FIGURE 20