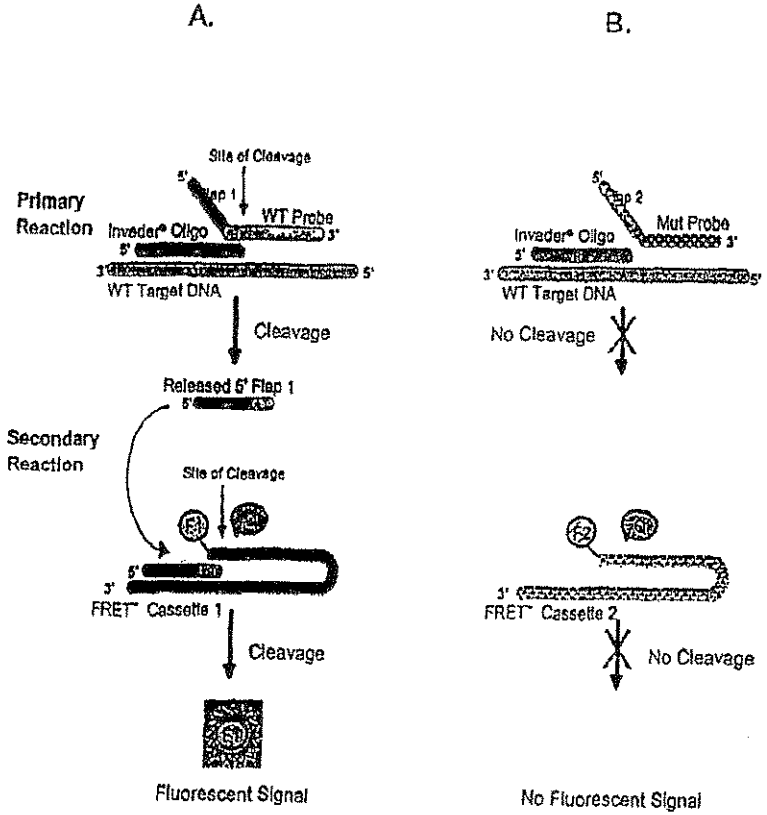

A.
B.
Site of Cleavage
Primary Reaction
Invader® Oligo
WT Probe
WT Target DNA
Cleavage
Released 5' Flap 1
Secondary Reaction
Site of Cleavage
FRET™ Cassette 1
Cleavage
Fluorescent Signal
Mut Probe
No Cleavage
FRET™ Cassette 2
No Cleavage
No Fluorescent Signal

Fig. 1

**Figure 2**

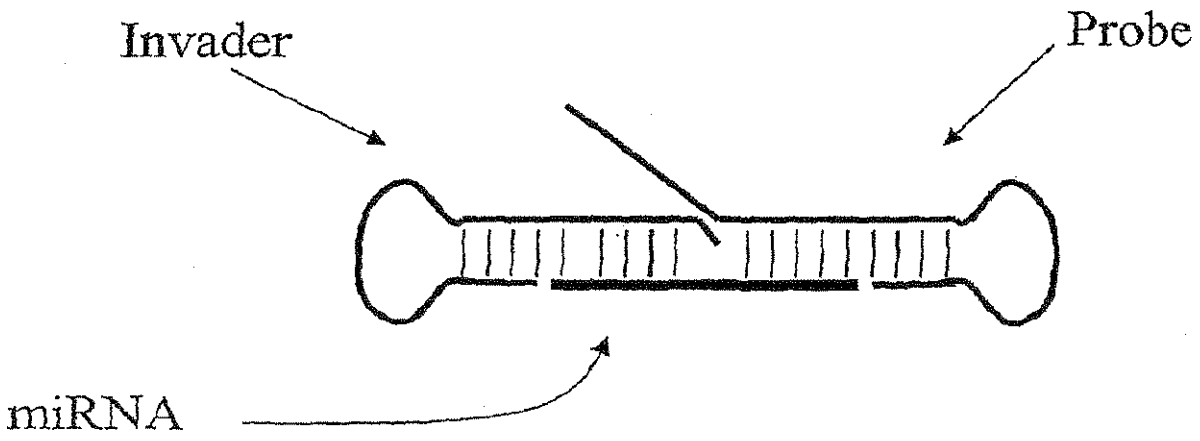
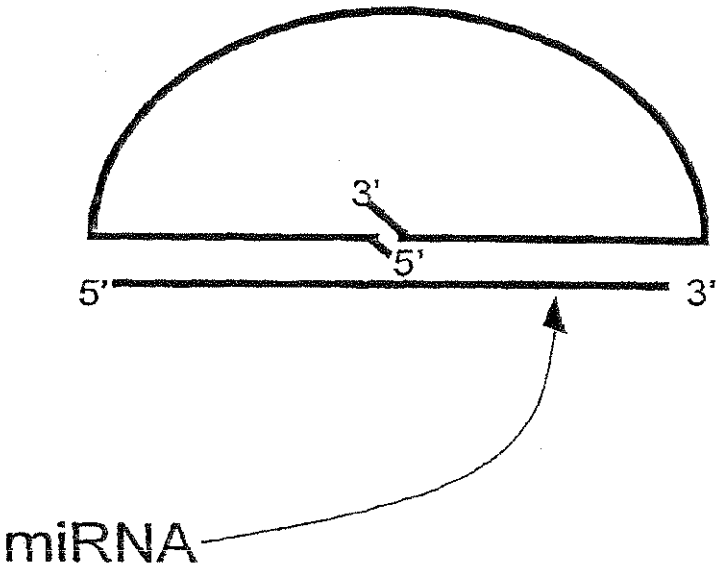
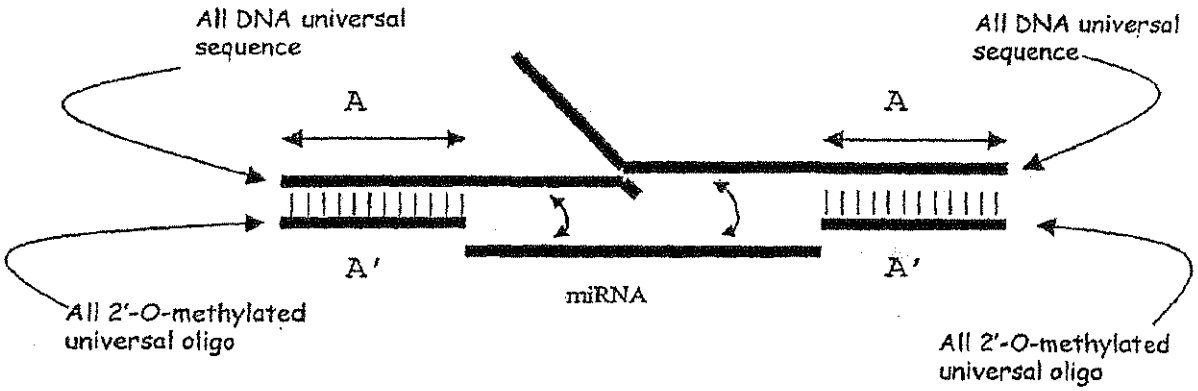


Figure 3



# Figure 4




A = Universal sequence that is added to the 3' and 5' of probes and INVADER oligonucleotides, respectively.
From 5' to 3', the probe is composed of the 5'-flap, the miRNA complementary region, and the DNA universal sequence "A".
The INVADER oligonucleotide from 5' to 3', is composed of the DNA universal sequence "A" and an miRNA complementary region.

A' = 2'-O-methyl universal oligonucleotide that compliments the sequence "A" and is added to kits as a standard oligonucleotide.

FIG. 5

| SEQ ID NO | Target | Oligo type | Sequence (5'-3') |
|---|---|---|---|
| SEQ ID NO:1 | human let-7 miRNA | Invader oligo | ggcacuuuugugccAACTATACAACCG |
| SEQ ID NO:2 | human let-7 miRNA | probe oligo | CCGTCGCTGCGTTACTACCTCAcgacguuuucgucg |
| SEQ ID NO:3 | human let-7 miRNA | arrestor oligo | cgacgaaaacgucgugagguaguaacgcag |
| SEQ ID NO:4 | human let-7 miRNA | miRNA | ugagguaguagguuguauaguu |
| SEQ ID NO:5 | human let-7 miRNA | Invader oligo | ggcacuuuugugccAACTATACAACT |
| SEQ ID NO:6 | human let-7 miRNA | probe oligo | CCGTCGCTGCGTCTACTACCTCAcgacguuuucgucg |
| SEQ ID NO:7 | human let-7 miRNA | arrestor oligo | cgacgaaaacgucgugagguaguagacgcag |
| SEQ ID NO:8 | human let-7 miRNA | Invader oligo | ggcacuuuugugccAACTATACAAT |
| SEQ ID NO:9 | human let-7 miRNA | probe oligo | AACGAGGCGCACCCTACTACCTCAcgacguuuucgucg |
| SEQ ID NO:10 | human let-7 miRNA | arrestor oligo | cgacgaaaacgucgugagguaguagggugcgc |
| SEQ ID NO:11 | human miR-1 | Invader oligo | ggcagcuuuugcugccCTCCATACTTCTC |
| SEQ ID NO:12 | human miR-1 | probe oligo | AACGAGGCGCACTTACATTCCAcgagccuuuuggcucg |
| SEQ ID NO:13 | human miR-1 | arrestor oligo | cgagccaaaaggcucguggaauguaagugcgc |
| SEQ ID NO:14 | human miR-1 | miRNA | uggaauguaaagaaguauggag |
| SEQ ID NO:15 | human miR-1 | Invader oligo | ggcagcuuuugcugccCTCCATACTTCC |
| SEQ ID NO:16 | human miR-1 | probe oligo | AACGAGGCGCACTTTACATTCCAcgagccuuuuggcucg |
| SEQ ID NO:17 | human miR-1 | arrestor oligo | cgagccaaaaggcucguggaauguaaagugcgc |
| SEQ ID NO:18 | human miR-1 | Invader oligo | ggcagcuuuugcugccCTCCATACTTT |
| SEQ ID NO:19 | human miR-1 | probe oligo | AACGAGGCGCACCTTTACATTCCAcgagccuuuuggcucg |
| SEQ ID NO:20 | human miR-1 | arrestor oligo | cgagccaaaaggcucguggaauguaaaggugcgc |
| SEQ ID NO:21 | FAM FRET | FRET probe | Yca-cXt-gct-tcg-tgg |
| SEQ ID NO:22 | SRT | Secondary Reaction template | CCA GGA AGC AAG TGA CGC AGC GAC ggu |
| SEQ ID NO:23 | human let-7 miRNA | Invader oligo | ggcacuuuugugccaaCTATACAAT |
| SEQ ID NO:24 | human let-7c miRNA | miRNA | uugguauguuggauggauggagu |
| SEQ ID NO:25 | human let-7e miRNA | miRNA | ugguacguuggauggauggagu |
| SEQ ID NO:26 | human let-7f miRNA | miRNA | uugauauguuagaugauggagu |
| SEQ ID NO:27 | human miR-135 | Invader oligo | ccgagcgaaagcucggTTCACATAGGAATC |
| SEQ ID NO:28 | human miR-135 | probe oligo | AACGAGGCGCACAAAAAGCCATAcgagccgaaaggcucg |
| SEQ ID NO:29 | human miR-135 | arrestor oligo | cgagccuuucggcucguauggcuuuuugugcgc |
| SEQ ID NO:30 | human miR-135 | Invader oligo | ccgagcgaaagcucggTTCACATAGGAAC |
| SEQ ID NO:31 | human miR-135 | probe oligo | AACGAGGCGCACTAAAAAGCCATAcgagccgaaaggcucg |
| SEQ ID NO:32 | human miR-135 | arrestor oligo | cgagccuuucggcucguauggcuuuuuagugcgc |
| SEQ ID NO:33 | human miR-135 | Invader oligo | ccgagcgaaagcucggTTCACATAGGAC |
| SEQ ID NO:34 | human miR-135 | probe oligo | AACGAGGCGCACATAAAAAGCCATAcgagccgaaaggcucg |
| SEQ ID NO:35 | human miR-135 | arrestor oligo | cgagccuuucggcucguauggcuuuuuaugugcgc |
| SEQ ID NO:36 | human miR-135 | Invader oligo | ccgagcgaaagcucggTTCACATAGGC |
| SEQ ID NO:37 | human miR-135 | probe oligo | AACGAGGCGCACAATAAAAAGCCATAcgagccgaaaggcucg |
| SEQ ID NO:38 | human miR-135 | arrestor oligo | cgagccuuucggcucguauggcuuuuuauugugcgc |

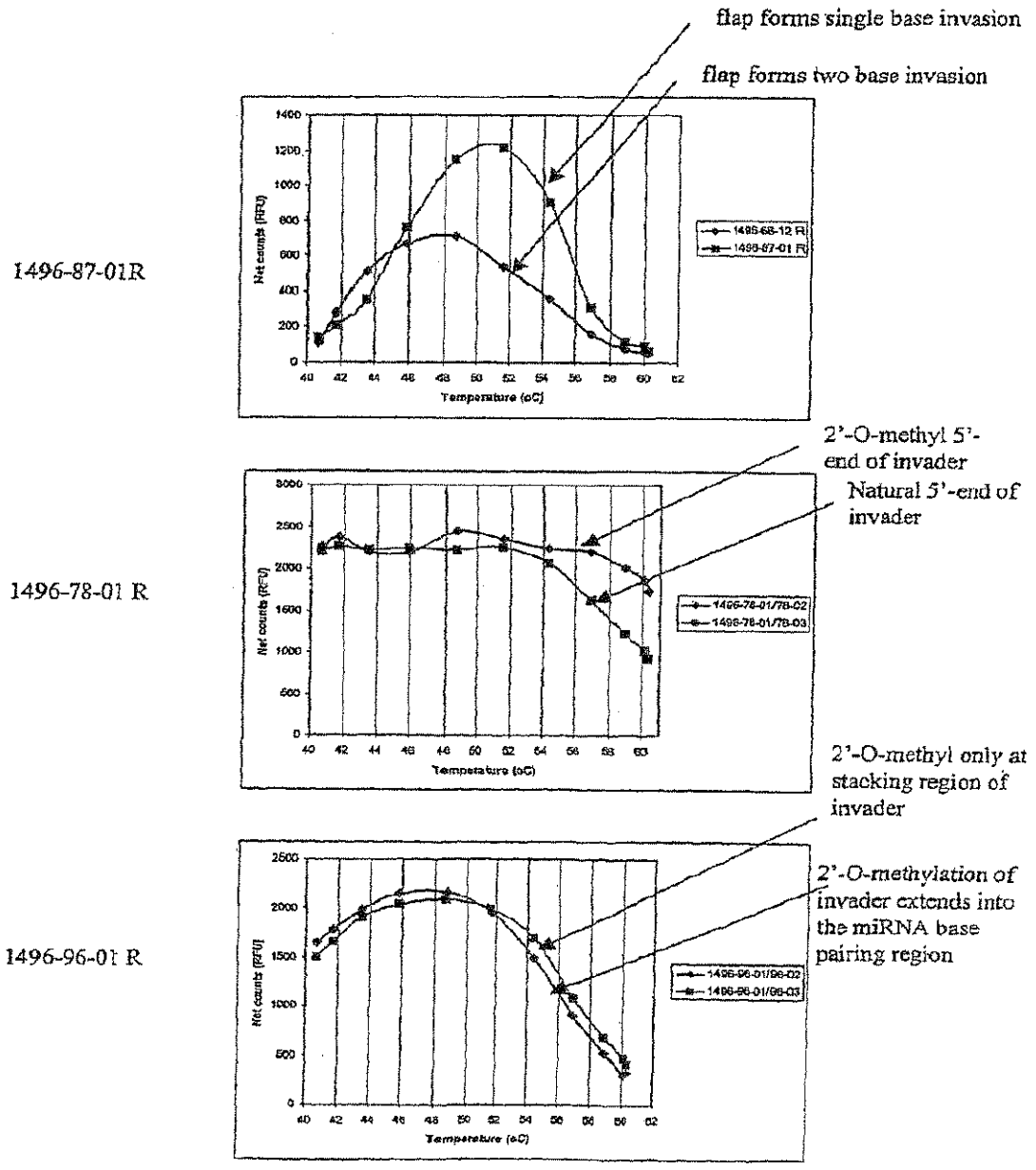
FIG. 5

| SEQ ID NO | Target | Oligo type | Sequence (5'-3') |
|---|---|---|---|
| SEQ ID NO:39 | human miR-16 | miRNA | uagcagcacgtaaauauuggcg |
| SEQ ID NO:40 | SRT | Secondary Reaction template | CCAGGAAGCAAGTGGAGGCGTGACggu |
| SEQ ID NO:41 | human GAPDH | Invader oligo | ggaaucauauuGGAACATGTAAACCATC |
| SEQ ID NO:42 | human GAPDH | probe oligo | CCGCCGAGATCACGTAGTTGAGGTC-NH2 |
| SEQ ID NO:43 | human GAPDH | arrestor oligo | gaccucaacuacgugauc |
| SEQ ID NO:44 | human miR-125b | miRNA | ucccugagacccuaacuuguga |
| SEQ ID NO:45 | U6 RNA | Invader oligo | GGCCATGCTAATCTTCA |
| SEQ ID NO:46 | U6 RNA | probe oligo | CCGCCGAGATCACTCTGTATCGTTC-NH2 |
| SEQ ID NO:47 | U6 RNA | arrestor oligo | gaacgauacagagugauc |
| SEQ ID NO:48 | RED FRET | | Ycl-cXl-tct-cag-tgc-g |
| SEQ ID NO:49 | SRT | Secondary Reaction template | CCAGCAAGCAAGTGGTGATCTCGGCggu |
| SEQ ID NO:50 | human let-7a miRNA | probe oligo | CCGTCGCTGCGTCTACTACCTCA-NH2 |
| SEQ ID NO:51 | human let-7a miRNA | Invader oligo | AACTATACAACT |
| SEQ ID NO:52 | human let-7a miRNA | probe oligo | CCGTCGCTGCGTTACTACCTCA-NH2 |
| SEQ ID NO:53 | human let-7a miRNA | Invader oligo | AACTATACAACCG |
| SEQ ID NO:54 | human let-7a miRNA | arrestor oligo | ugagguaguagacgcag |
| SEQ ID NO:55 | human miR-15 | probe oligo | AACGAGGCGCACATGTGCTGCTAcgagccuuuuggcucg |
| SEQ ID NO:56 | human miR-15 | Invader oligo | ggcagcuuuugcugccCACAAACCATTC |
| SEQ ID NO:57 | human miR-15 | arrestor oligo | cgagccaaaaggcucguagcagcacaugugcgc |
| SEQ ID NO:58 | human miR-15 | probe oligo | AACGAGGCGCACATGTGCTGCTAGCTCGCCACGCCG-NH2 |
| SEQ ID NO:59 | human miR-15 | Invader oligo | GCTCGCCACGCCGCACAAACCATTC |
| SEQ ID NO:60 | human miR-15 | stacker oligo | cggcguggcgagc |
| SEQ ID NO:61 | human miR-15 | arrestor oligo | cggcguggcgagcuagcagcacaugugcgc |
| SEQ ID NO:62 | human miR-15 | miRNA | uagcagcacauaaugguuugug |
| SEQ ID NO:63 | human miR-135 | probe oligo | AACGAGGCGCACAATAAAAAGCCATAGCTCGCCACGCCG-NH2 |
| SEQ ID NO:64 | human miR-135 | Invader oligo | GCTCGCCACGCCGTTCACATAGGC |
| SEQ ID NO:65 | human miR-135 | arrestor oligo | cggcguggcgagcuauggcuuuuuauugugcgc |
| SEQ ID NO:66 | human miR-15 | arrestor oligo | uagcagcacaugugcgc |
| SEQ ID NO:67 | human miR-15 | probe oligo | AACGAGGCGCACATGTGCTGCTAGGCGAAGCC |
| SEQ ID NO:68 | human miR-15 | Invader oligo | GGCGAAGCCCACAAACCATTC |
| SEQ ID NO:69 | human miR-15 | probe oligo | AACGAGGCGCACATGTGCTGCTAGGCGAAgcc |
| SEQ ID NO:70 | human miR-15 | Invader oligo | ggcGAAGCCCACAAACCATTC |
| SEQ ID NO:71 | human miR-15 | probe oligo | AACGAGGCGCACATGTGCTGCTAggcuucggcc |
| SEQ ID NO:72 | human miR-15 | Invader oligo | ggcuucggccCACAAACCATTC |
| SEQ ID NO:73 | human let-7a miRNA | Invader oligo | GGCACTTTTGTGCCAACTATACAACT |
| SEQ ID NO:74 | human let-7a miRNA | probe oligo | CCGTCGCTGCGTCTACTACCTCACGACGTTTTCGTCG |
| SEQ ID NO:75 | human let-7a miRNA | Invader oligo | ggcacTTTTGTGCCAACTATACAACT |
| SEQ ID NO:76 | human let-7a miRNA | probe oligo | CCGTCGCTGCGTCTACTACCTCACGACGTTTTcg'ucg |

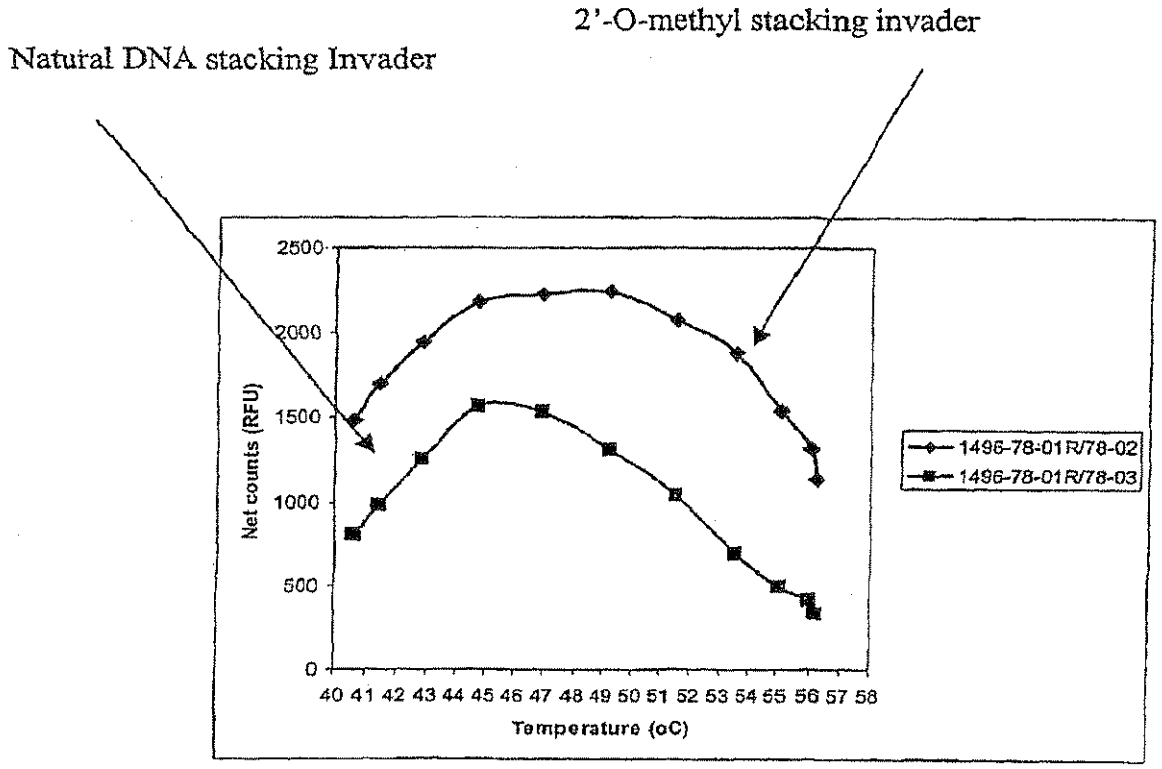
FIG. 5

| SEQ ID NO | Target | Oligo type | Sequence (5'-3') |
|---|---|---|---|
| SEQ ID NO:77 | human miR-16 miRNA | Invader oligo | ggcagcuuuugcugccCGCCAATATTG |
| SEQ ID NO:78 | human miR-16 miRNA | probe oligo | AACGAGGCGCACTACGTGCTGCTAcgagccuuuuggcucg |
| SEQ ID NO:79 | human miR-16 miRNA | arrestor oligo | cgagccaaaaggcucguagcagcacguagugcgc |
| SEQ ID NO:80 | human miR-125b miRNA | Invader oligo | ggcagcuuuugcugccTCACAAGTTAGA |
| SEQ ID NO:81 | human miR-125b miRNA | probe oligo | AACGAGGCGCACGGTCTCAGGGAcgagccuuuuggcucg |
| SEQ ID NO:82 | human miR-125b miRNA | arrestor oligo | cgagccaaaaggcucgucccugagaccgugcgc |
| SEQ ID NO:83 | human let-7a miRNA | probe oligo | CCGTCGCTGCGTCTACTACCTCAcgacguuuucgucgu |
| SEQ ID NO:84 | human let-7a miRNA | Invader oligo | uggcacuuuugugccAACTATACAACT |
| SEQ ID NO:85 | human let-7a miRNA | probe oligo | CCGTCGCTGCGTCTACTACCTCAcgacguuuucguc |
| SEQ ID NO:86 | human let-7a miRNA | Invader oligo | gcacuuuugugccAACTATACAACT |
| SEQ ID NO:87 | precursor human let-7a | miRNA | gggcuuuggggugagguaguaggguuguauaguuuggaauauuaccaccggugaacuau gcaauuuucuaccuuuccugaaguccc |
| SEQ ID NO:88 | miR-124a 21nt | miRNA | uaaggcacgcggugaaugcca |
| SEQ ID NO:89 | miR-124a 22nt | miRNA | uuaaggcacgcggugaaugcca |
| SEQ ID NO:90 | miR-124a miRNA | probe oligo | CCGTCGCTGCGTCGCGTGCCTTAcgagccuuuuggcucg |
| SEQ ID NO:91 | miR-124a miRNA | arrestor oligo | uaaggcacgcgacgcag |
| SEQ ID NO:92 | miR-124a miRNA | Invader oligo | ggcagcuuuugcugccTGGCATTCACA |
| SEQ ID NO:93 | U6 RNA | probe oligo | CCGCCGAGATCACCTAATCTTCTCTGTAT-NH2 |
| SEQ ID NO:94 | U6 RNA | Invader oligo | CATCCTTGCGCAGGGGCCATGA |
| SEQ ID NO:95 | U6 RNA | arrestor oligo | auacagagaagauuaggugauc |
| SEQ ID NO:96 | human miR-135 | miRNA | uauggcuuuuuauuccuaugugaa |
| SEQ ID NO:97 | human miR-1d | miRNA | uggaauguaaagaaguauguau |
| SEQ ID NO:98 | human miR-1d | probe oligo | AACGAGGCGCACTTTACATTCCAcgagccuuuuggcucg |
| SEQ ID NO:99 | human miR-1d | Invader oligo | ggcagcuuuugcugccATACATACTTCC |
| SEQ ID NO:100 | human miR-1d | arrestor oligo | cgagccaaaaggcucguggaauguaaagugcgc |
| SEQ ID NO:101 | human beta actin siRNA | probe oligo-antisense | AACGAGGCGCACAAGATCATTGCggcuucggcc |
| SEQ ID NO:102 | human beta actin siRNA | Invader oligo-antisense | ggcuucggccAATGAAGATCC |
| SEQ ID NO:103 | human beta actin siRNA | arrestor oligo-antisense | gcaaugaucuugugcgc |
| SEQ ID NO:104 | human beta actin siRNA | probe oligo-sense | AACGAGGCGCACCTTGATCTTCAggcuucggcc |
| SEQ ID NO:105 | human beta actin siRNA | Invader oligo-sense | ggcuucggccAAGCAATGATA |
| SEQ ID NO:106 | human beta actin siRNA | arrestor oligo-sense | ugaagaucaaggugcgc |

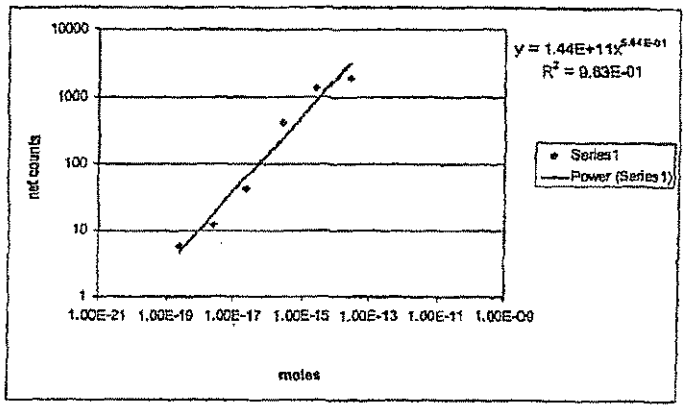
# Figure 6
# Design Optimization

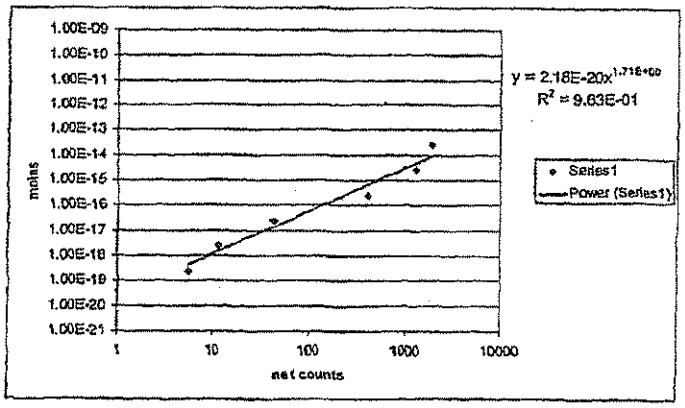


# Figure 7
# Design Optimization



# Figure 8
# LOD let-7 (1496-78-01R)



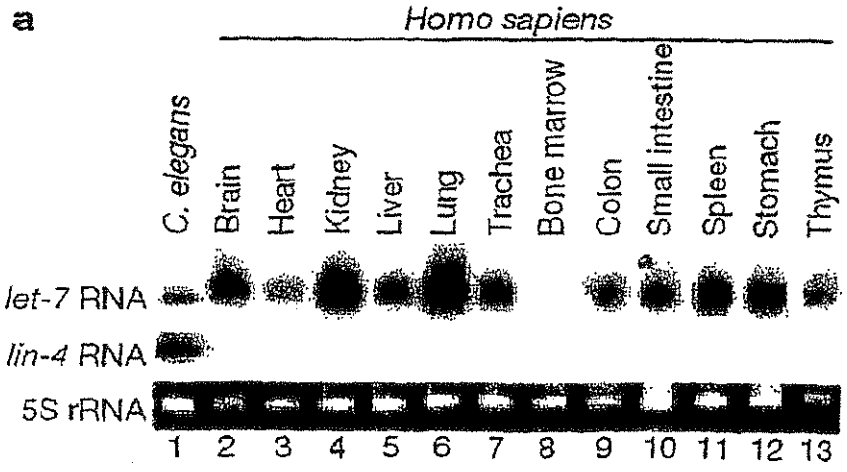
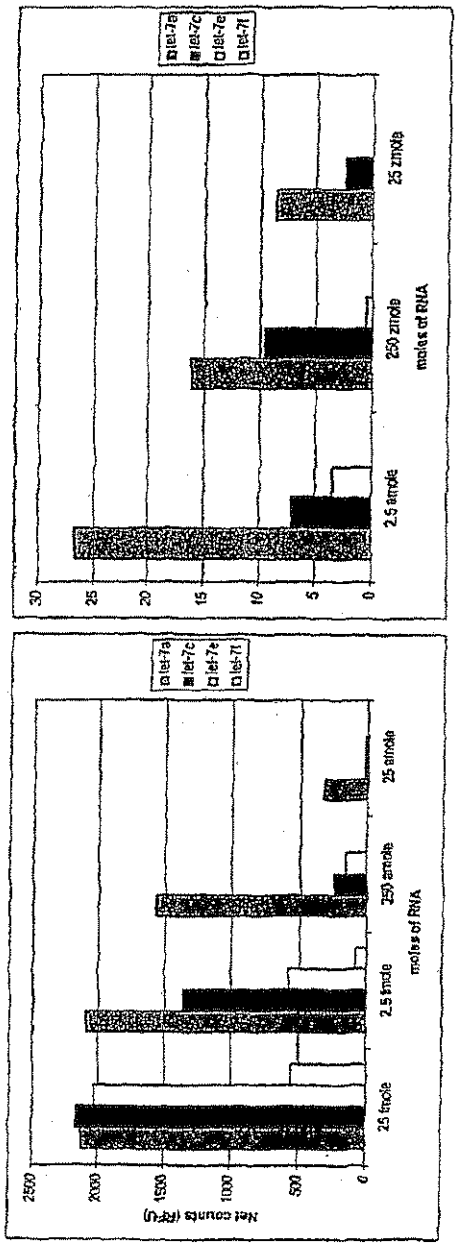
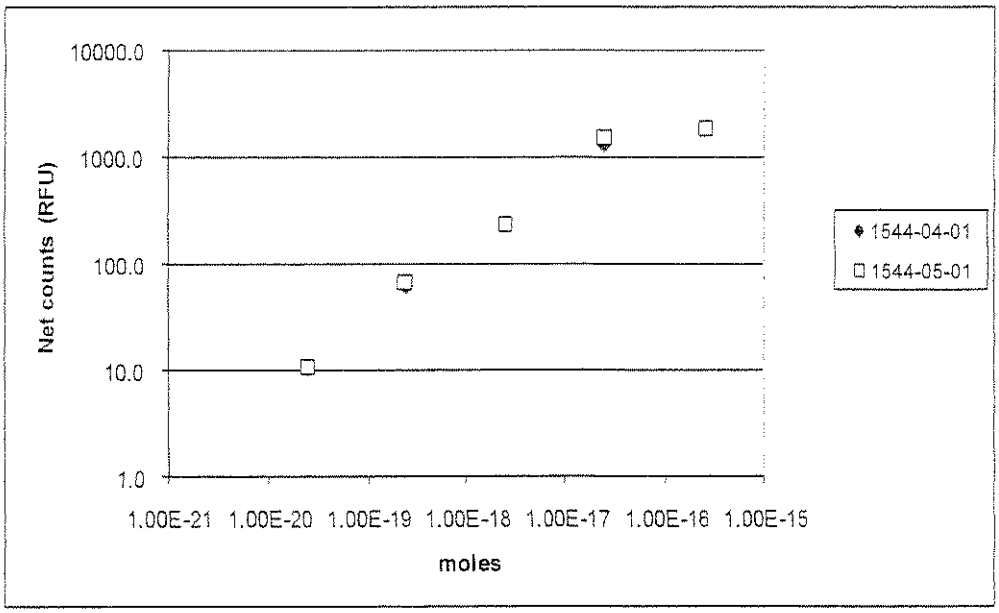



Figure 9
cross reactivity let-7



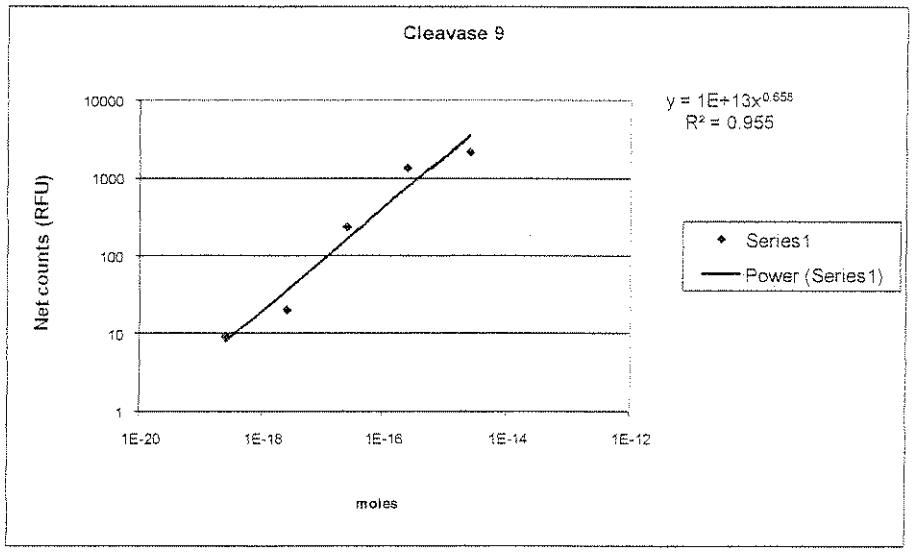
# Figure 10

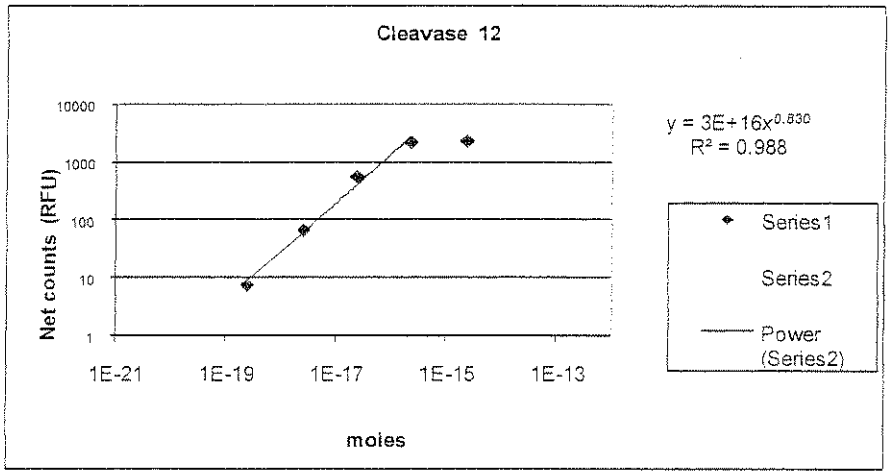
## LOD mir-1



# Figure 11

## LOD let-7 (1496-78-01R) using CLEAVASE XII enzyme



# FIGURE 12

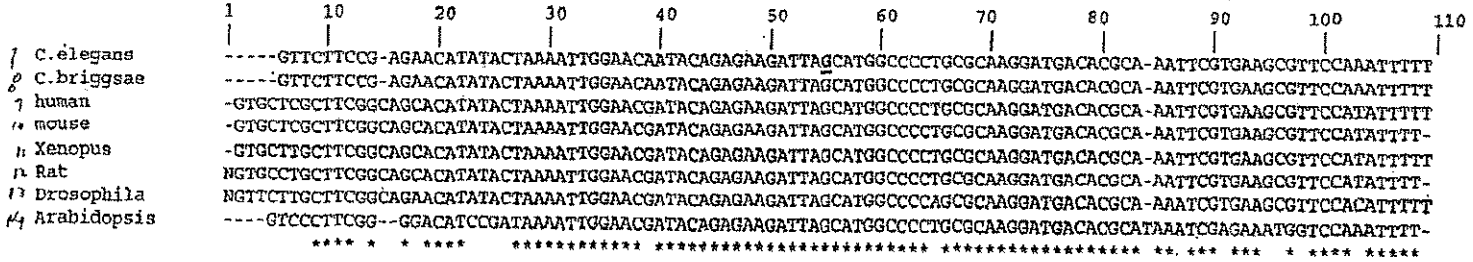
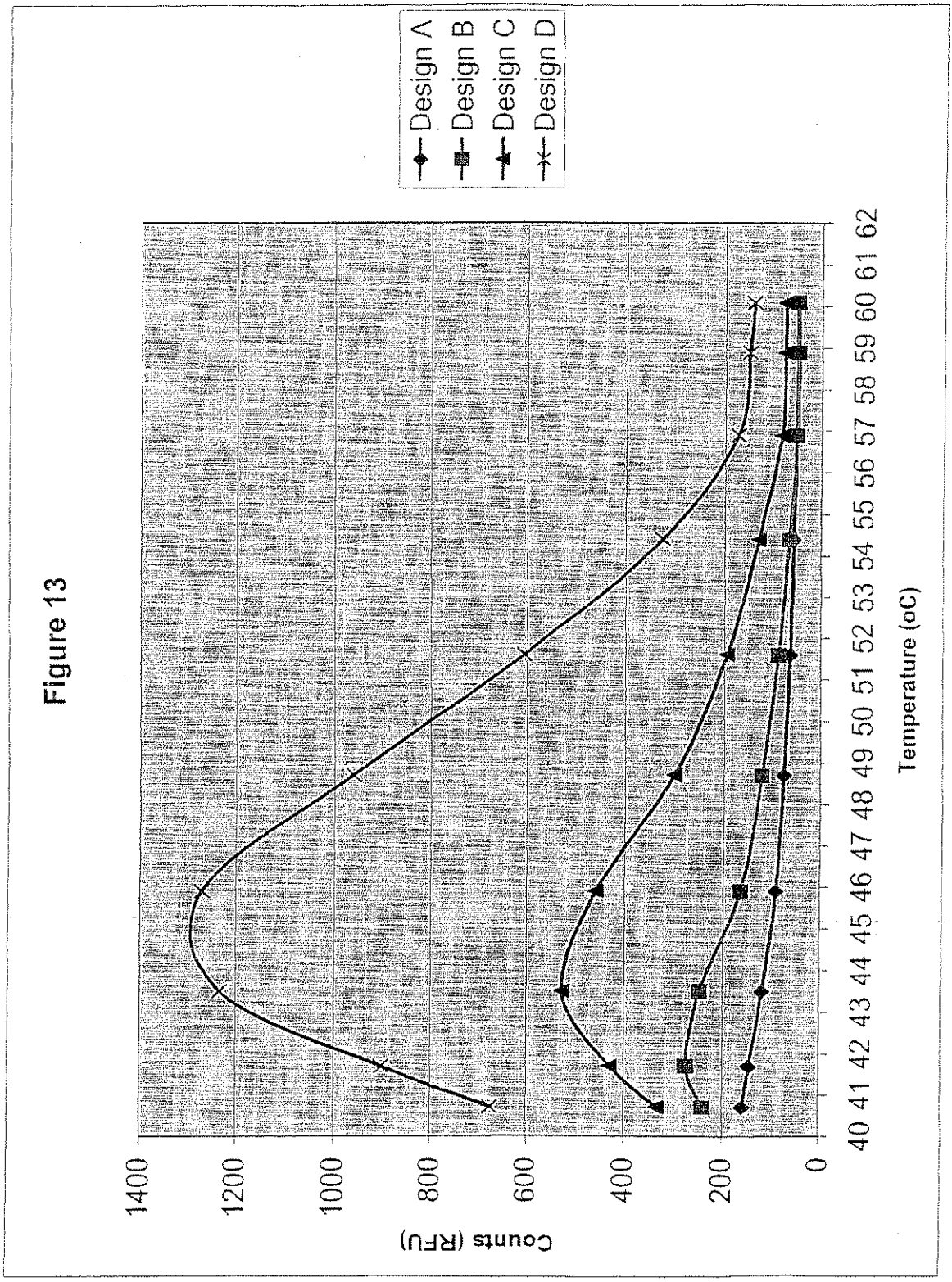

1 10 20 30 40 50 60 70 80 90 100 110
C.elegans -----GTTCTTCCG-AGAACATATACTAAAATTGGAACAATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCAAATTTTT
C.briggsae -----GTTCTTCCG-AGAACATATACTAAAATTGGAACAATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCAAATTTTT
human -GTGCTCGCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCATATTTTT
mouse -GTGCTCGCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCATATTTT-
Xenopus -GTGCTTGCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCATATTTTT
Rat NGTGCCTGCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCATATTTT-
Drosophila NGTTCTTGCTTCGGCAGAACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCAGCGCAAGGATGACACGCA-AAATCGTGAAGCGTTCCACATTTTT
Arabidopsis ----GTCCCTTCGG--GGACATCCGATAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCATAAATCGAGAAATGGTCCAAATTTT-

Figure 13


Design A
Design B
Design C
Design D
Counts (RFU)
1400
1200
1000
800
600
400
200
0
40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62
Temperature (oC)

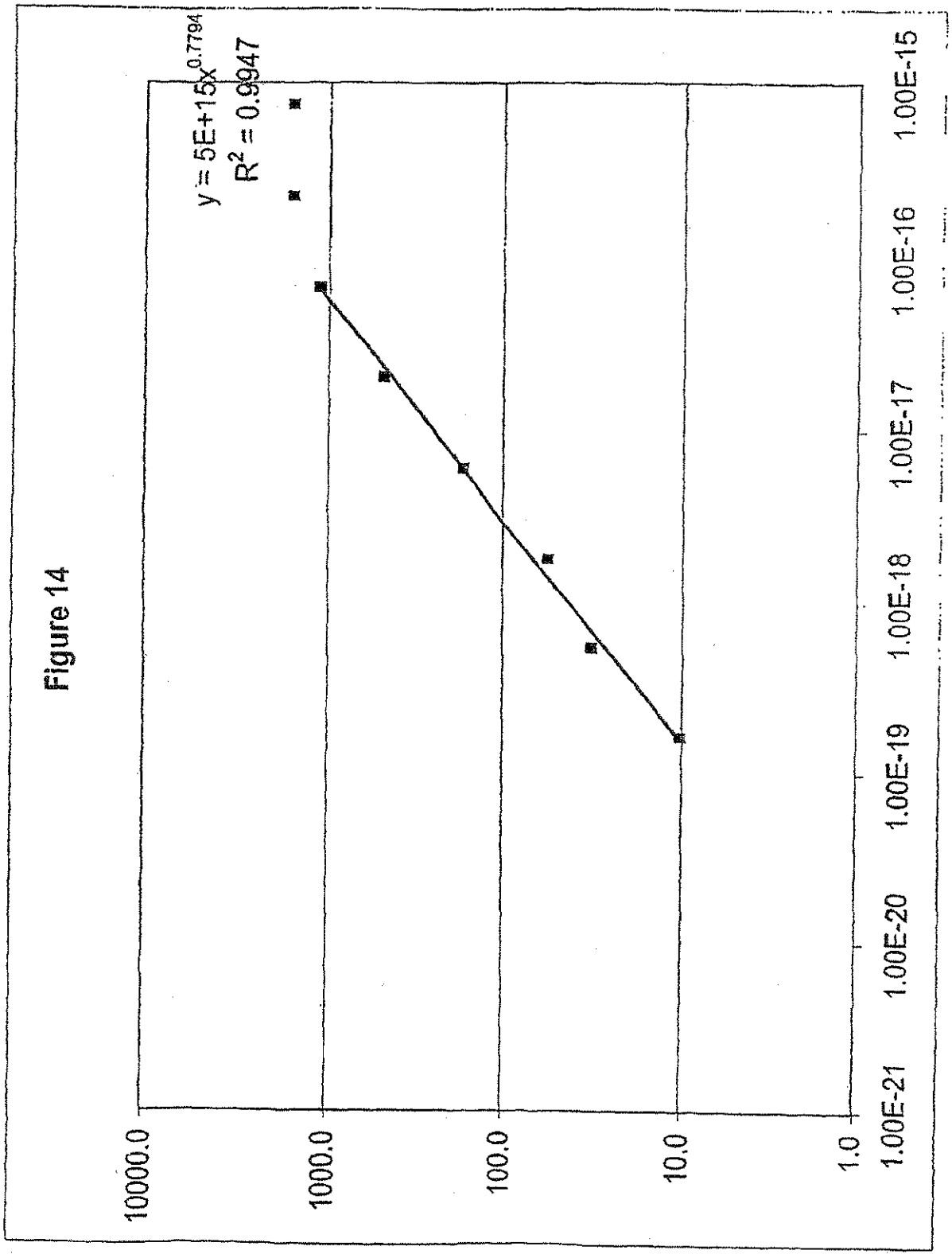

y = 5E+15x^-0.7794
R² = 0.9947
10000.0
1000.0
100.0
10.0
1.0
1.00E-21
1.00E-20
1.00E-19
1.00E-18
1.00E-17
1.00E-16
1.00E-15

Figure 14

Fig. 15

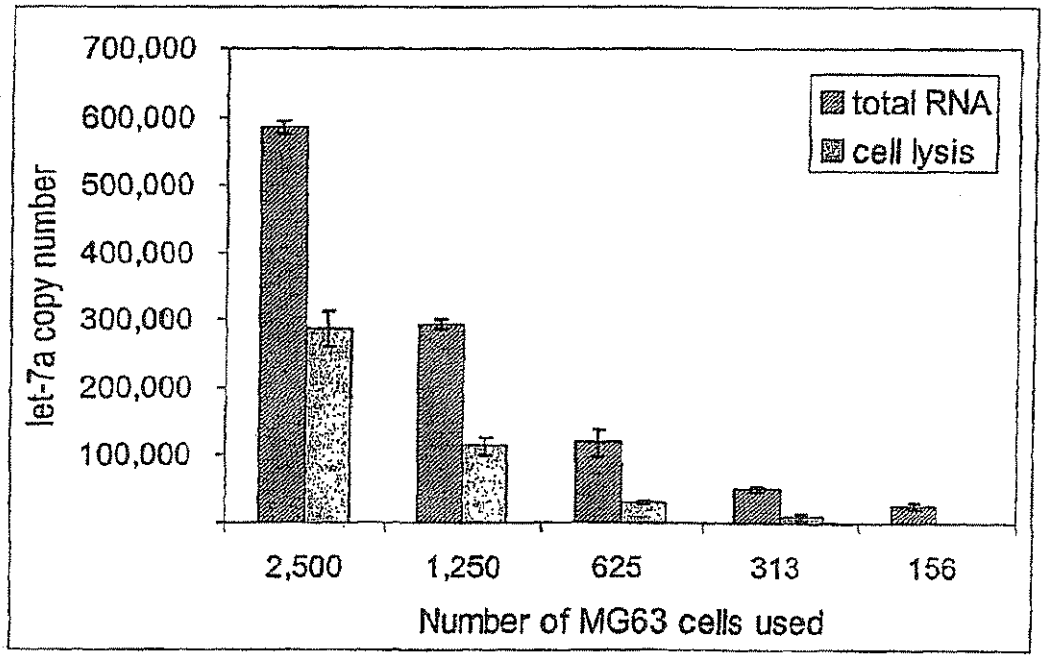

700,000
600,000
500,000
400,000
300,000
200,000
100,000
let-7a copy number
total RNA
cell lysis
2,500
1,250
625
313
156
Number of MG63 cells used

Figure 16

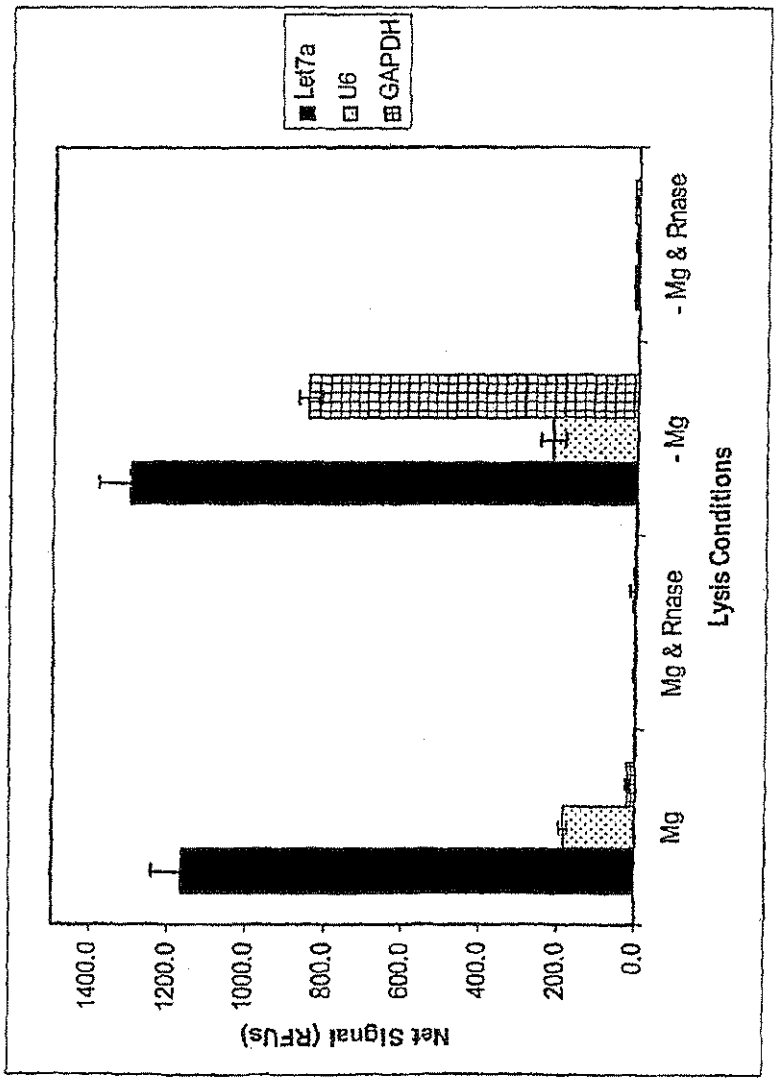
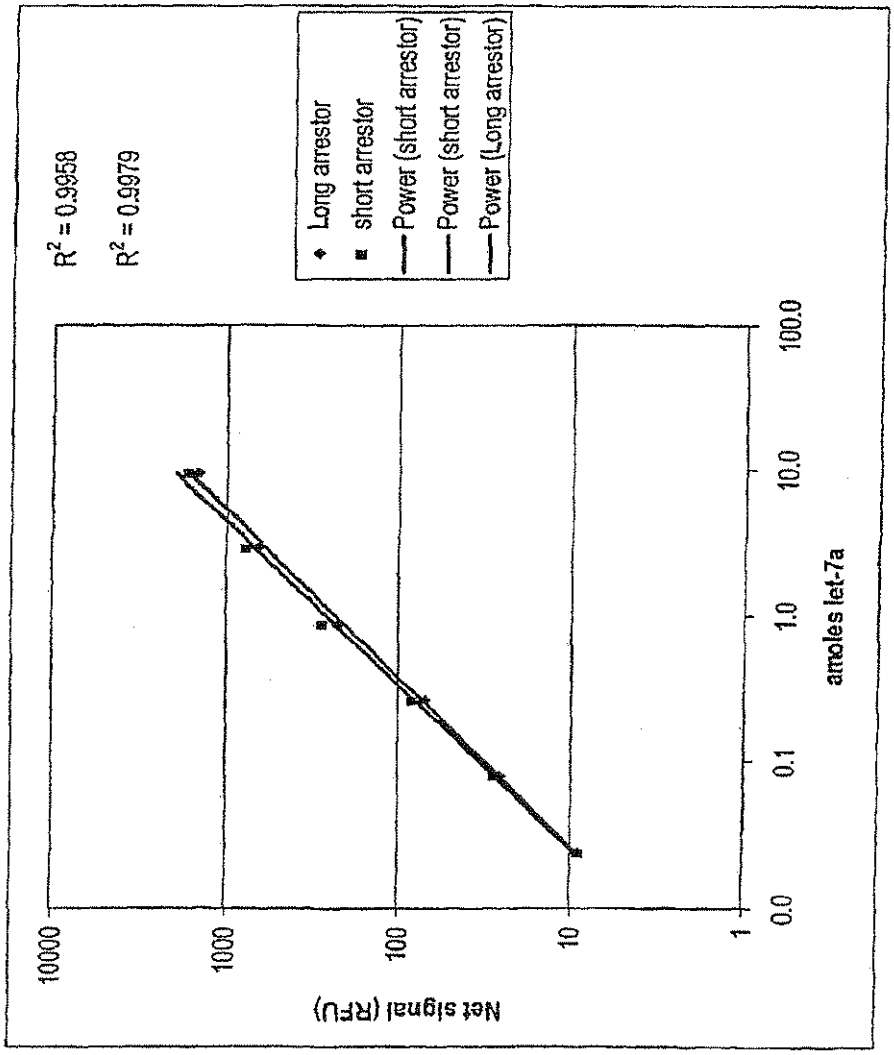
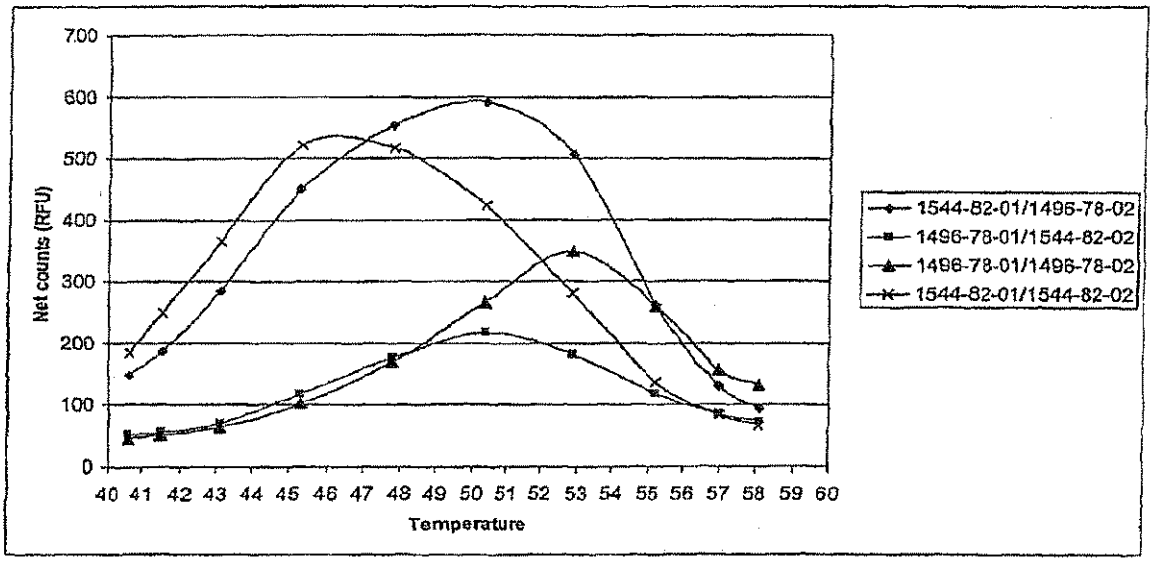
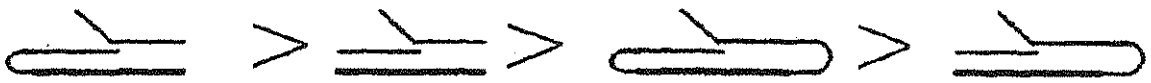

Let7a
U6
GAPDH
Net Signal (RFUs)
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Mg
Mg & Rnase
- Mg
- Mg & Rnase
Lysis Conditions

Figure 17


Long arrestor
short arrestor
Power (short arrestor)
Power (short arrestor)
Power (Long arrestor)
R2 = 0.9958
R2 = 0.9979
Net signal (RFU)
10000
1000
100
10
1
0.0
0.1
1.0
10.0
100.0
amoles let-7a

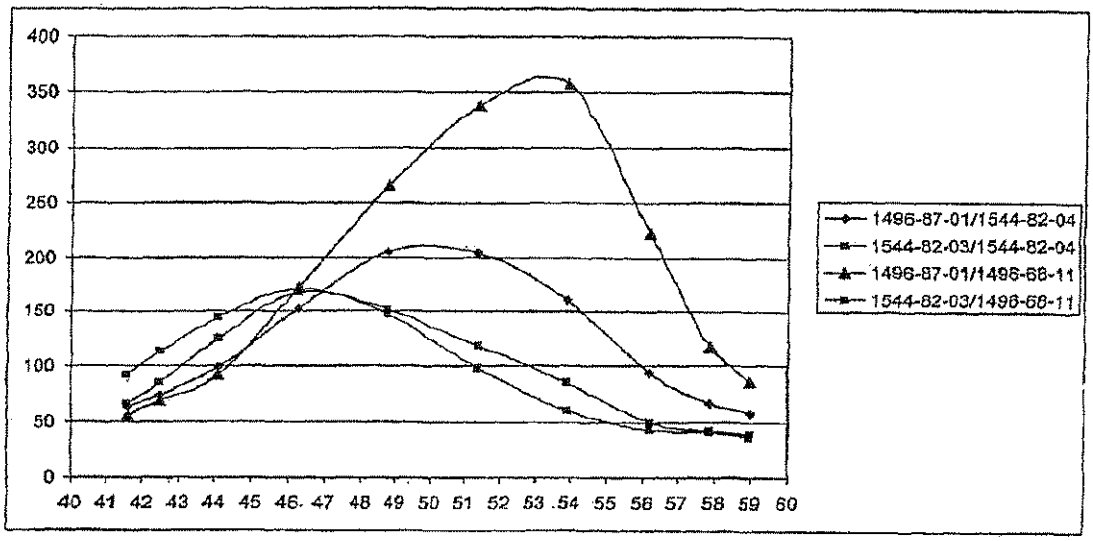
# FIGURE 18

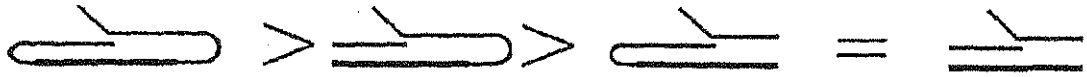


| | | | | |
|---|---|---|---|---|
| Normalized maximum performance | 100% | 90% | 60% | 30% |
| optimum temperature at maximum performance | 50 °C | 46 °C | 53 °C | 50 °C |

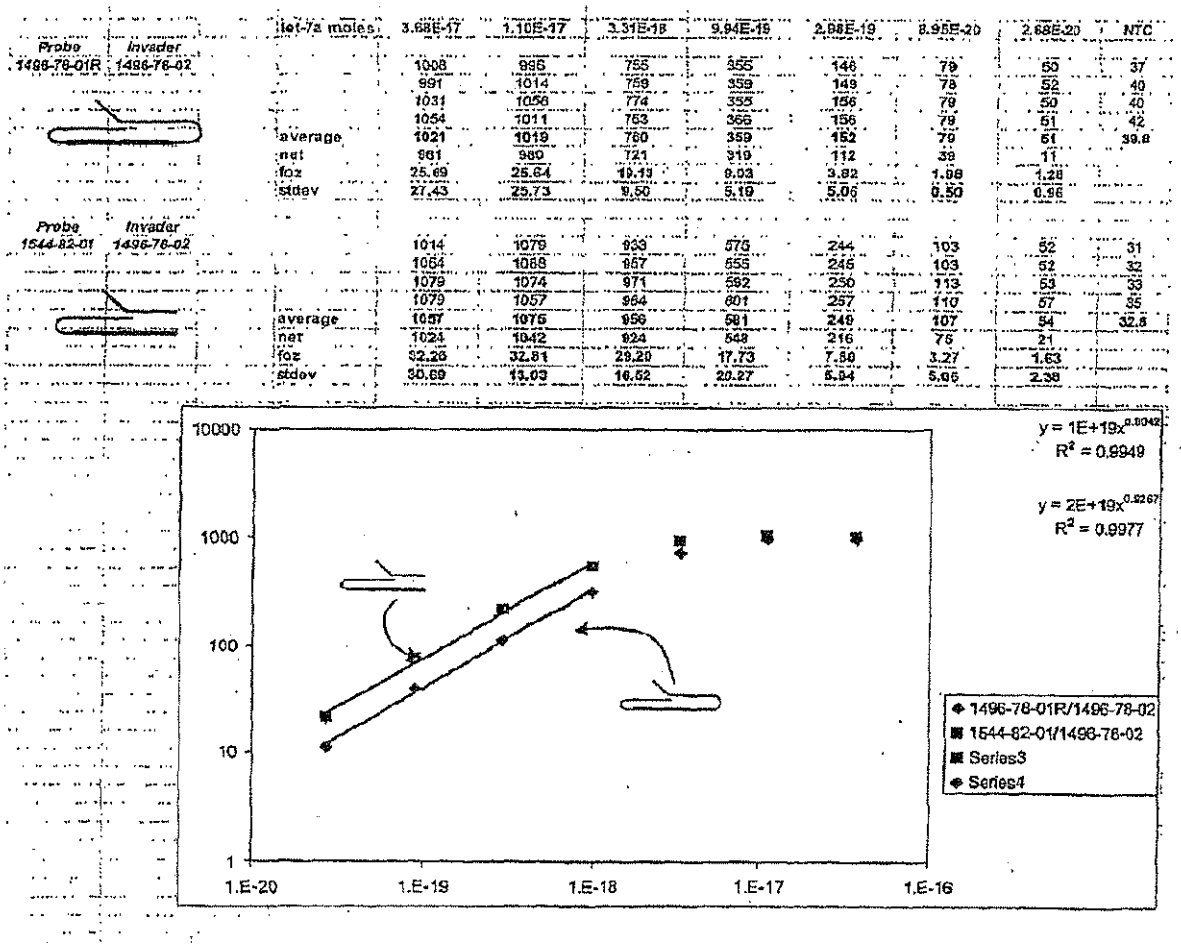
Fig. 19



| | | | | |
|---|---|---|---|---|
| Normalized maximum performance → | 100% | 60% | 50% | 50% |
| optimum temperature at maximum performance → | 53 °C | 51 °C | 47 °C | 46 °C |

Fig. 20


let-7a moles
3.68E-17
1.10E-17
3.31E-18
9.94E-19
2.98E-19
8.95E-20
2.68E-20
NTC
Probe
1496-78-01R
Invader
1496-78-02
1008
996
755
355
146
79
60
37
991
1014
769
359
149
78
52
40
1031
1056
774
355
156
79
50
40
1054
1011
763
366
156
79
51
42
average
1021
1019
760
359
152
79
51
39.8
net
961
980
721
319
112
39
11
fox
25.69
26.64
19.19
9.03
3.82
1.98
1.28
stdev
27.43
25.73
9.50
5.19
5.06
0.50
0.96
Probe
1544-82-01
Invader
1496-78-02
1014
1079
933
575
244
103
52
31
1064
1068
957
555
246
103
52
32
1079
1074
971
592
250
113
53
33
1079
1057
964
601
257
110
57
35
average
1057
1075
956
581
249
107
54
32.8
net
1024
1042
924
548
216
75
21
fox
32.28
32.81
29.20
17.73
7.59
3.27
1.63
stdev
30.69
13.03
16.52
20.27
5.94
5.06
2.38
10000
1000
100
10
1
1.E-20
1.E-19
1.E-18
1.E-17
1.E-16
y = 1E+19x^0.9942
R² = 0.9949
y = 2E+19x^0.9267
R² = 0.9977
1496-78-01R/1496-78-02
1544-82-01/1496-78-02
Series3
Series4

# Figure 21

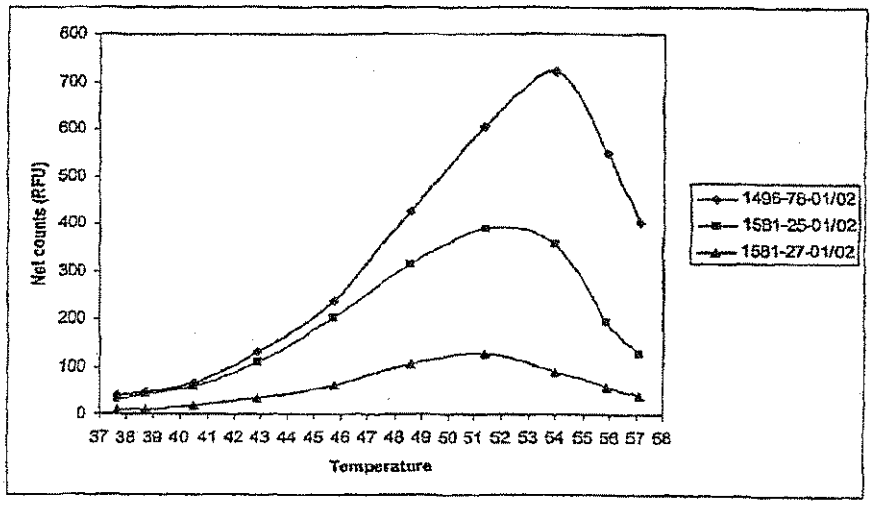
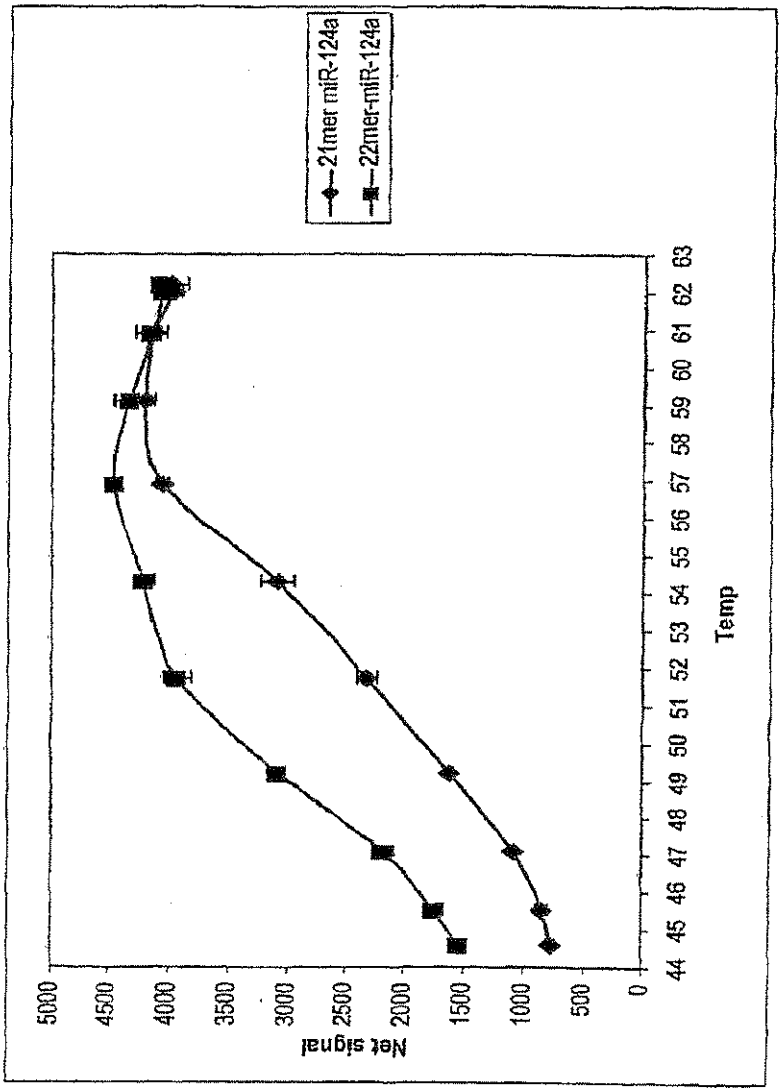

Net counts (RFU)
600
700
600
500
400
300
200
100
0
37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58
Temperature
1496-76-01/02
1581-25-01/02
1581-27-01/02

Figure 22


21mer miR-124a
22mer-miR-124a
Net signal
Temp
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63

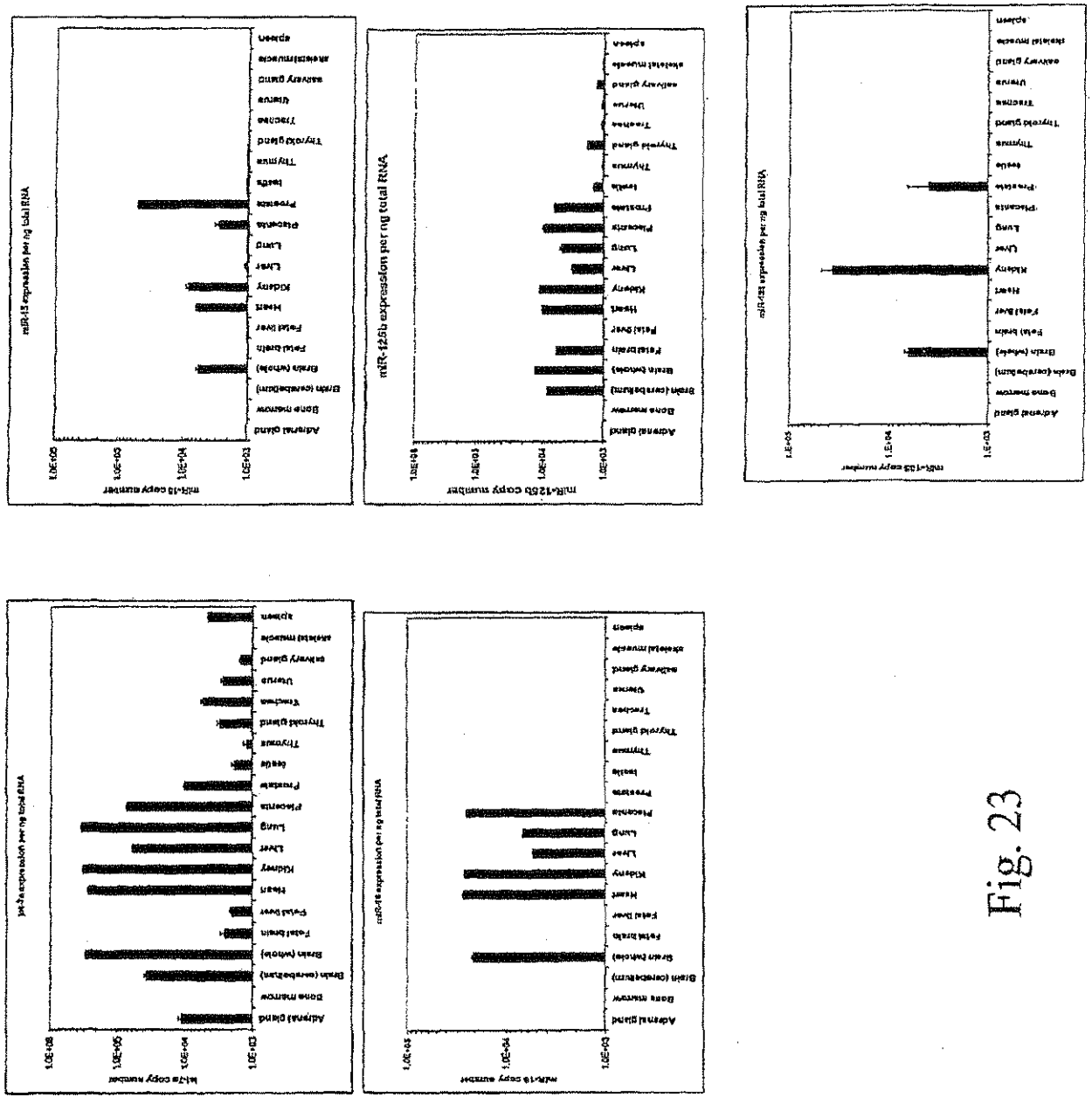
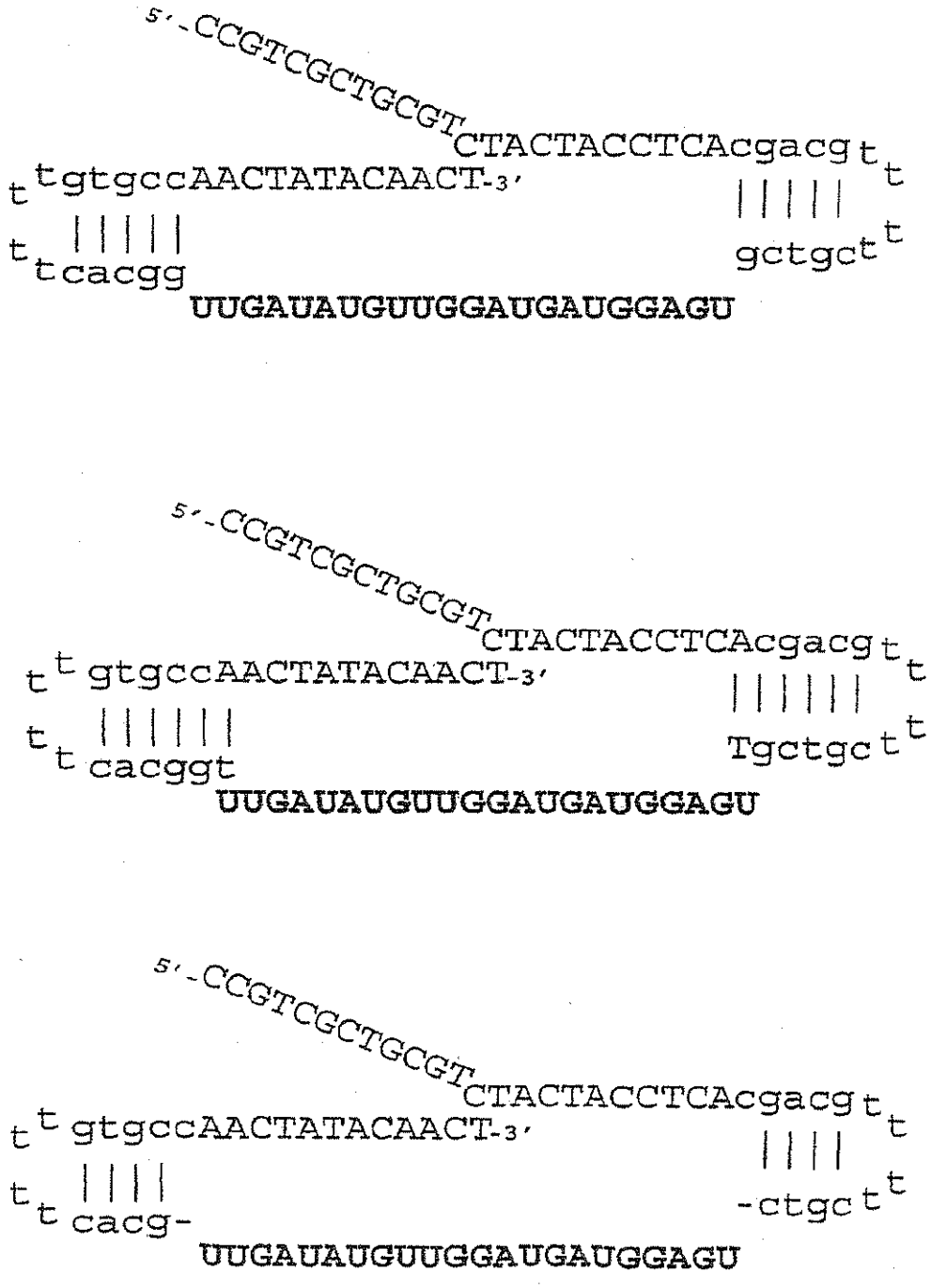


Fig. 23

Fig. 24


5'-CCGTCGCTGCGTCTACTACCTCAcgacg
ttgtgccAACTATACAACT-3'
ttcacgg
gctgcttt
UUGAUAUGUUGGAUGAUGGAGU
5'-CCGTCGCTGCGTCTACTACCTCAcgacg
ttgtgccAACTATACAACT-3'
ttcacggt
Tgctgcttt
UUGAUAUGUUGGAUGAUGGAGU
5'-CCGTCGCTGCGTCTACTACCTCAcgacg
ttgtgccAACTATACAACT-3'
ttcacg-
-ctgcttt
UUGAUAUGUUGGAUGAUGGAGU

# Figure 25

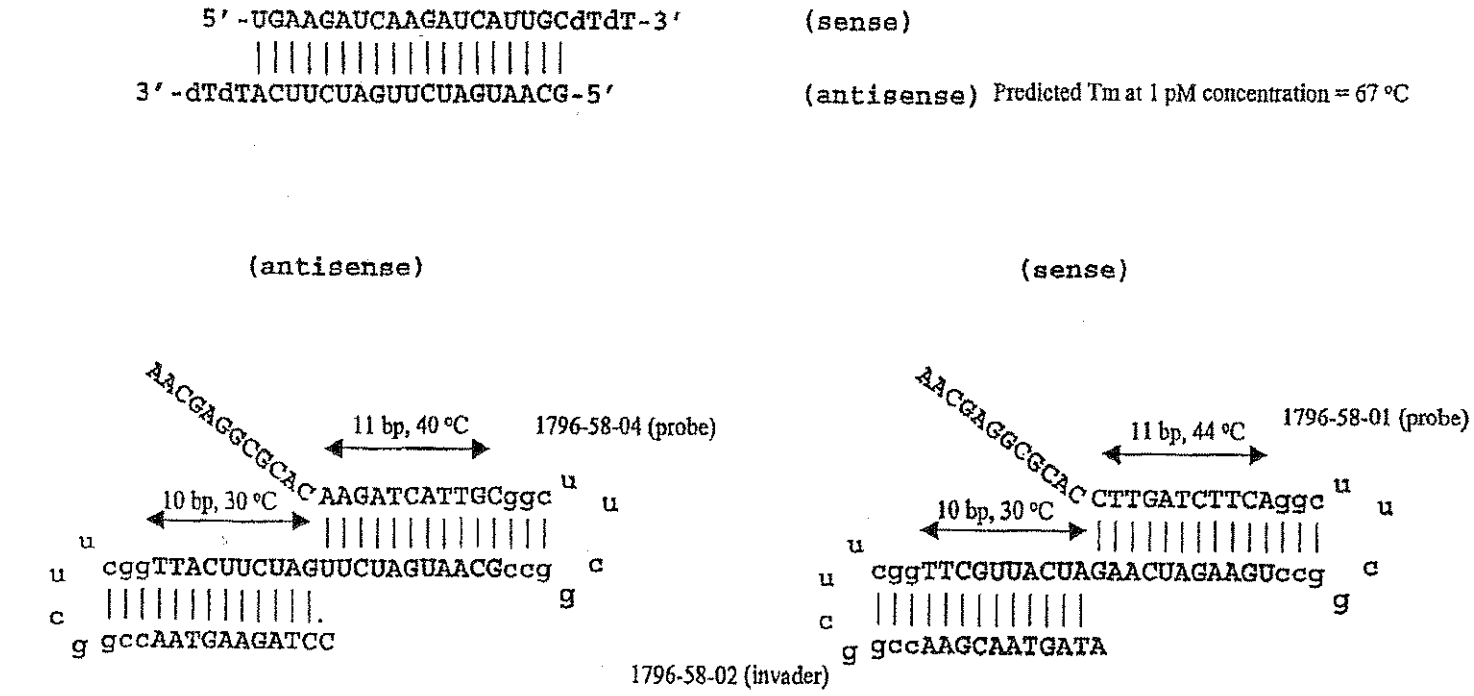
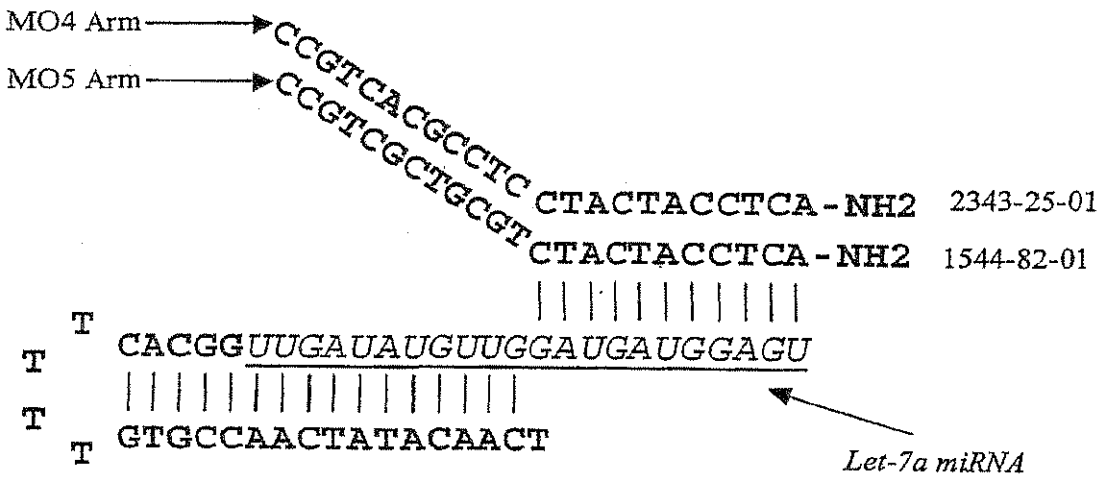

5′-UGAAGAUCAAGAUCAUUGCdTdT-3′
(sense)
3′-dTdTACUUCUAGUUCUAGUAACG-5′
(antisense)
Predicted Tm at 1 pM concentration = 67 °C
(antisense)
(sense)
AACGAGGCGCAC
11 bp, 40 °C
1796-58-04 (probe)
10 bp, 30 °C
AAGATCATTGCggc
cggTTACUUCUAGUUCUAGUAACGccg
gccAATGAAGATCC
1796-58-02 (invader)
AACGAGGCGCAC
11 bp, 44 °C
1796-58-01 (probe)
10 bp, 30 °C
CTTGATCTTCAggc
cggTTCGUUACUAGAACUAGAAGUccg
gccAAGCAATGATA
1796-58-05 (invader)
gcaaugaucuugugcgc
1796-58-06 (arrestor)
ugaagaucaaggugcgc
1796-58-03 (arrestor)

**FIGURE 26**




Invader oligo 1496-78-02

FIGURE 27

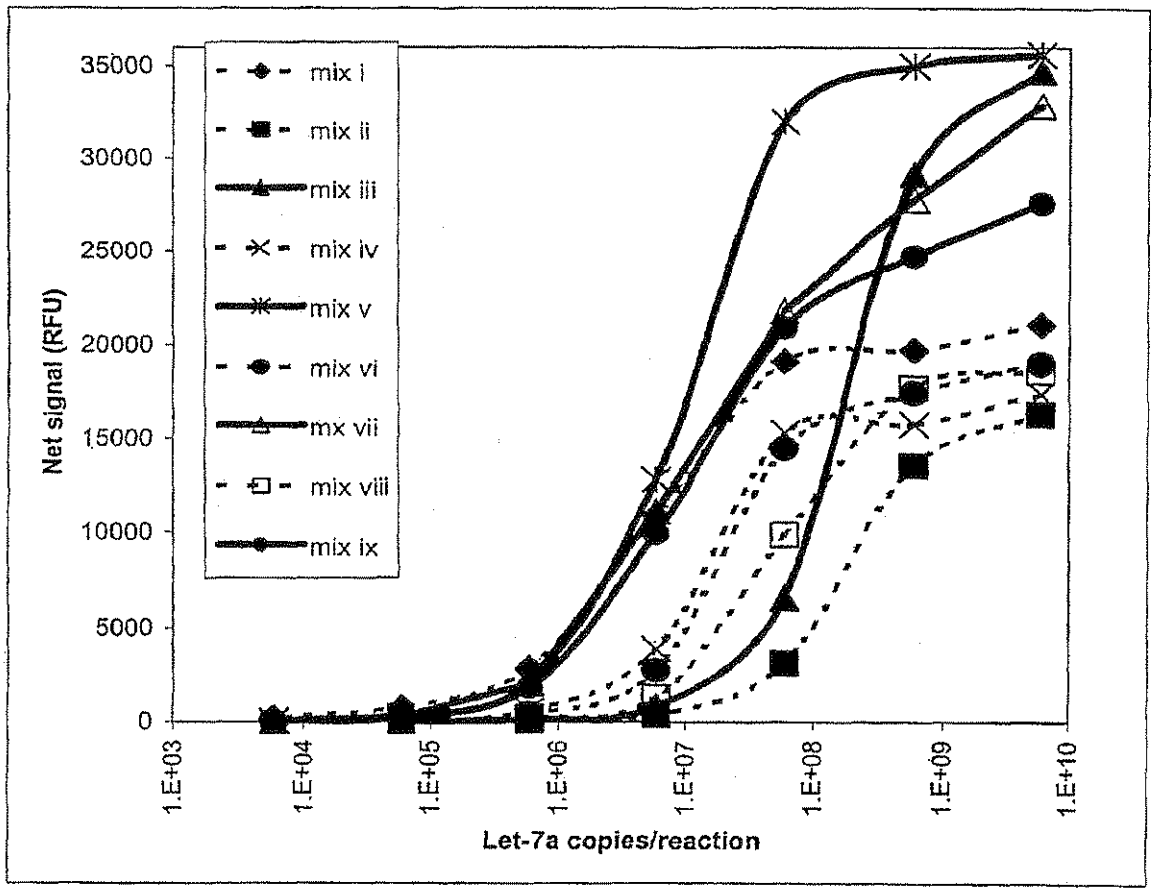

35000
30000
25000
20000
15000
10000
5000
0
Net signal (RFU)
mix i
mix ii
mix iii
mix iv
mix v
mix vi
mx vii
mix viii
mix ix
1.E+03
1.E+04
1.E+05
1.E+06
1.E+07
1.E+08
1.E+09
1.E+10
Let-7a copies/reaction

**FIGURE 28**

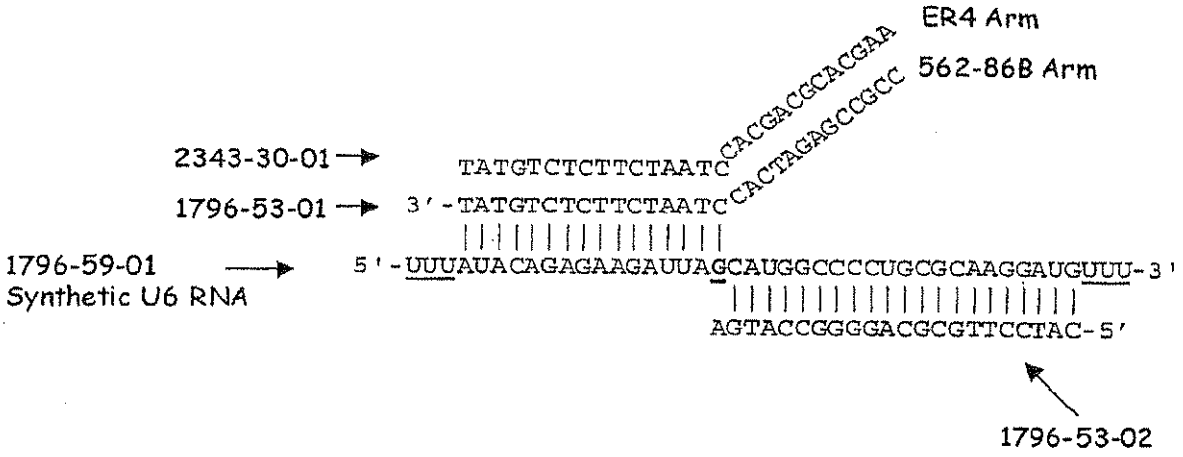

ER4 Arm
562-86B Arm
2343-30-01
TATGTCTCTTCTAATC
CACGACGCACGAA
CACTAGAGCCGCC
1796-53-01
3'-TATGTCTCTTCTAATC
1796-59-01
Synthetic U6 RNA
5'-UUUAUACAGAGAAGAUUAGCAUGGCCCCUGCGCAAGGAUGUUU-3'
AGTACCGGGGACGCGTTCCTAC-5'
1796-53-02

FIGURE 29

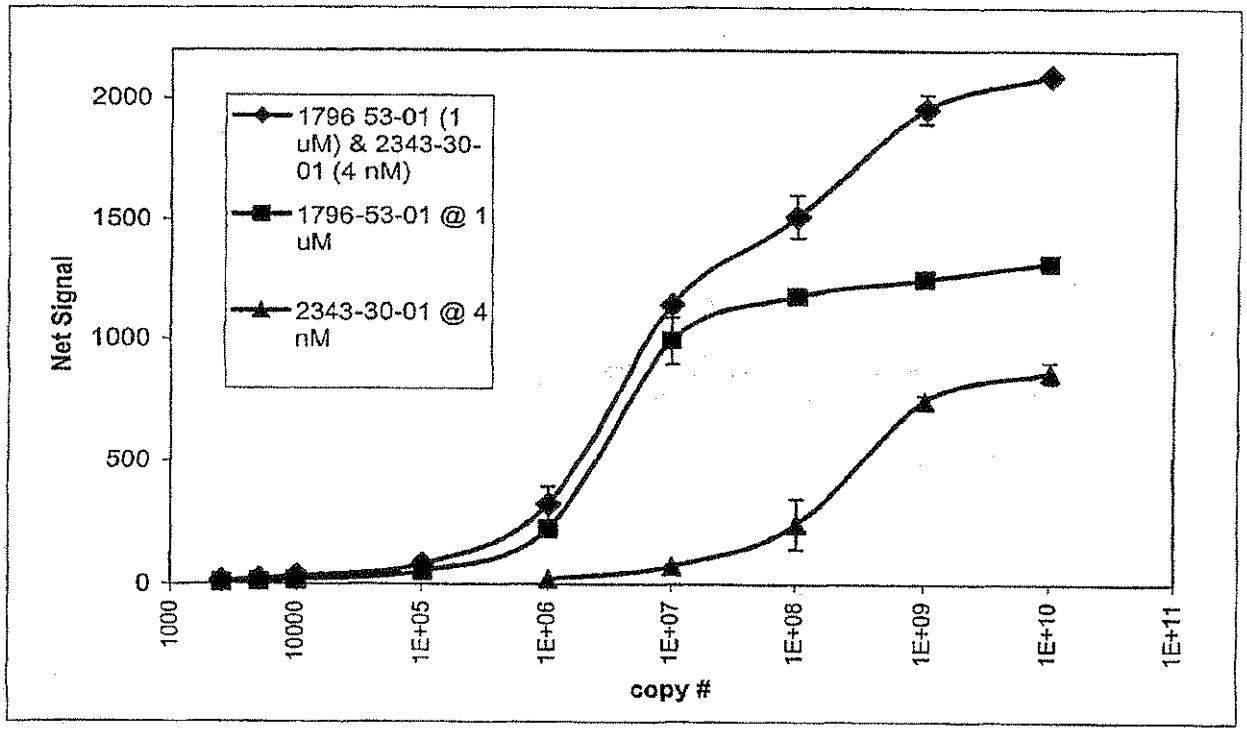

2000
1500
1000
500
0
Net Signal
1796 53-01 (1 uM) & 2343-30-01 (4 nM)
1796-53-01 @ 1 uM
2343-30-01 @ 4 nM
1000
10000
1E+05
1E+06
1E+07
1E+08
1E+09
1E+10
1E+11
copy #

**FIGURE 30**

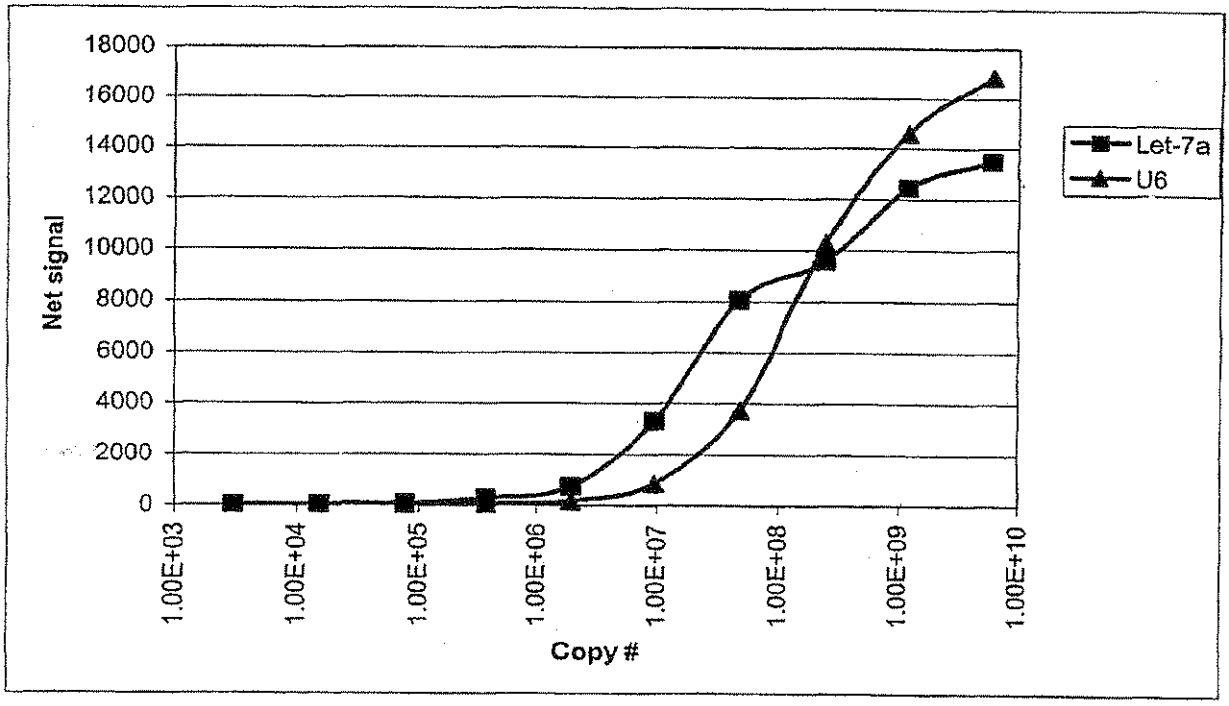

18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Net signal
1.00E+03
1.00E+04
1.00E+05
1.00E+06
1.00E+07
1.00E+08
1.00E+09
1.00E+10
Copy #
Let-7a
U6

**FIGURE 31**

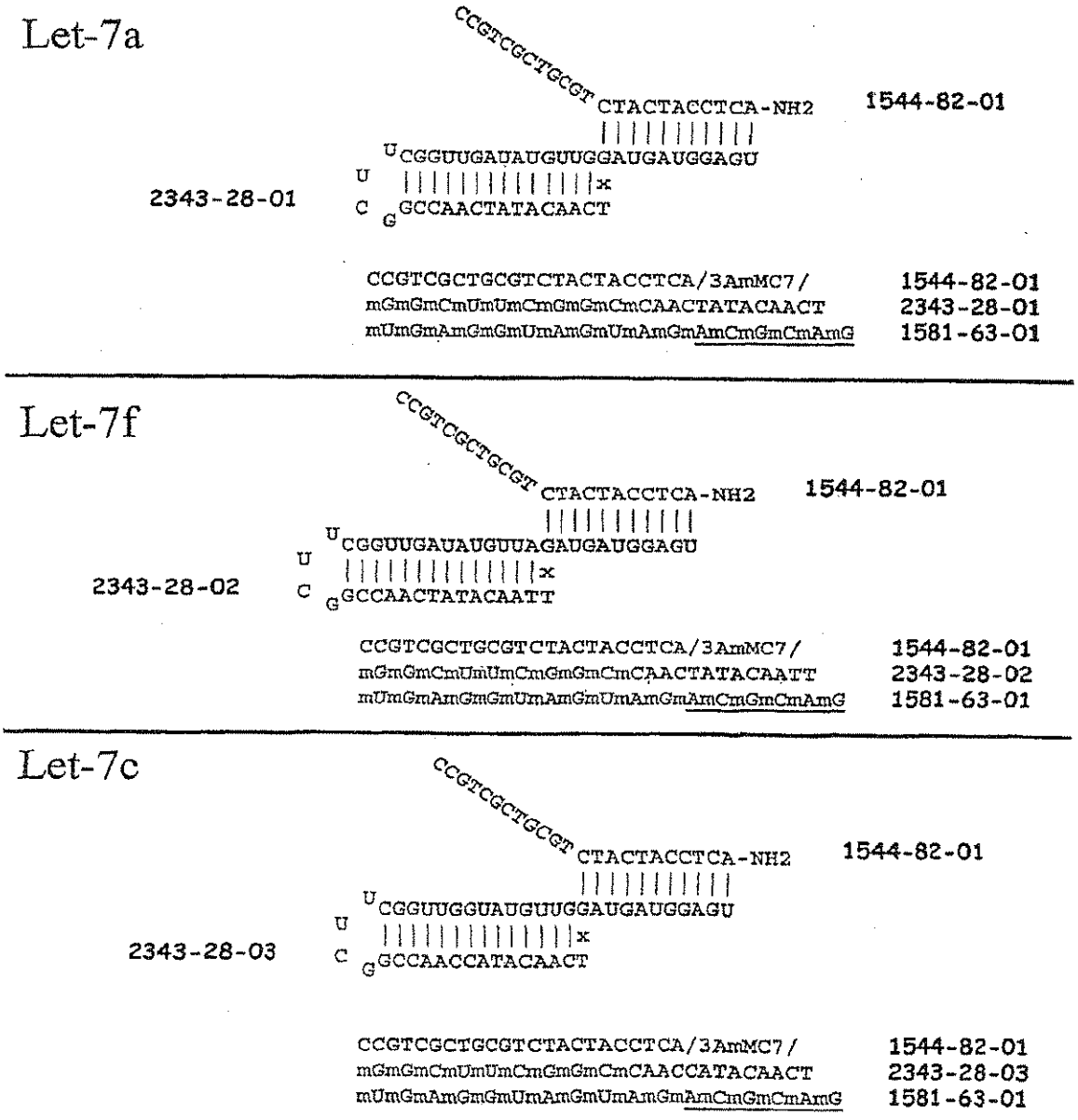
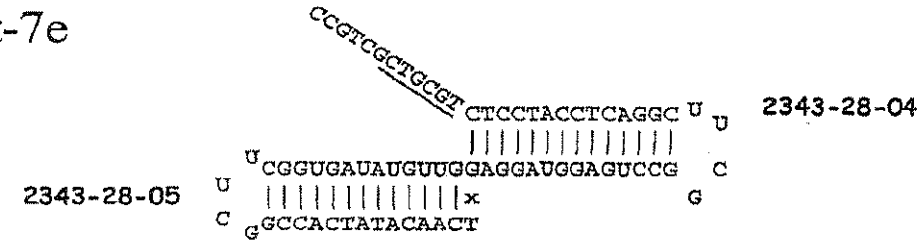

Let-7a
CCGTCGCTGCGT
CTACTACCTCA-NH2
1544-82-01
UCGGUUGAUAUGUUGGAUGAUGGAGU
U
C
G
GCCAACTATACAACT
2343-28-01
CCGTCGCTGCGTCTACTACCTCA/3AmMC7/ 1544-82-01
mGmGmCmUmUmCmGmGmCmCAACTATACAACT 2343-28-01
mUmGmAmGmGmUmAmGmUmAmGmAmCmGmCmAmG 1581-63-01
Let-7f
CCGTCGCTGCGT
CTACTACCTCA-NH2
1544-82-01
UCGGUUGAUAUGUUAGAUGAUGGAGU
U
C
G
GCCAACTATACAATT
2343-28-02
CCGTCGCTGCGTCTACTACCTCA/3AmMC7/ 1544-82-01
mGmGmCmUmUmCmGmGmCmCAACTATACAATT 2343-28-02
mUmGmAmGmGmUmAmGmUmAmGmAmCmGmCmAmG 1581-63-01
Let-7c
CCGTCGCTGCGT
CTACTACCTCA-NH2
1544-82-01
UCGGUUGGUAUGUUGGAUGAUGGAGU
U
C
G
GCCAACCATACAACT
2343-28-03
CCGTCGCTGCGTCTACTACCTCA/3AmMC7/ 1544-82-01
mGmGmCmUmUmCmGmGmCmCAACCATACAACT 2343-28-03
mUmGmAmGmGmUmAmGmUmAmGmAmCmGmCmAmG 1581-63-01

**FIGURE 31**
**Continued**

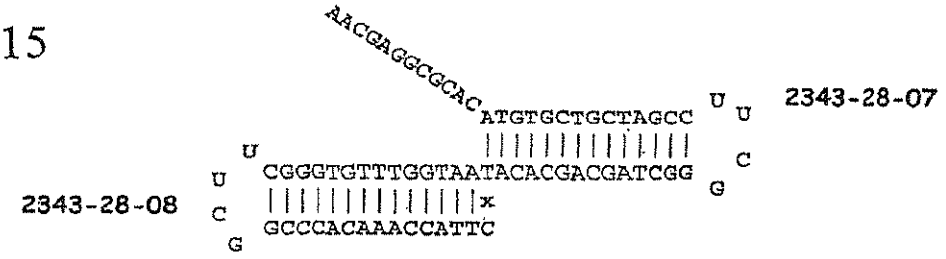
Let-7e




| | |
|---|---|
| CCGTCGCTGCGTCTCCTACCTCAmGmGmCmUmUmCmGmGmCmC | 2343-28-04 |
| mGmGmCmUmUmCmGmGmCmCACTATACAACT | 2343-28-05 |
| mUmGmAmGmGmUmAmGmGmAmGmAmCmGmCmAmG | 2343-28-06 |

---

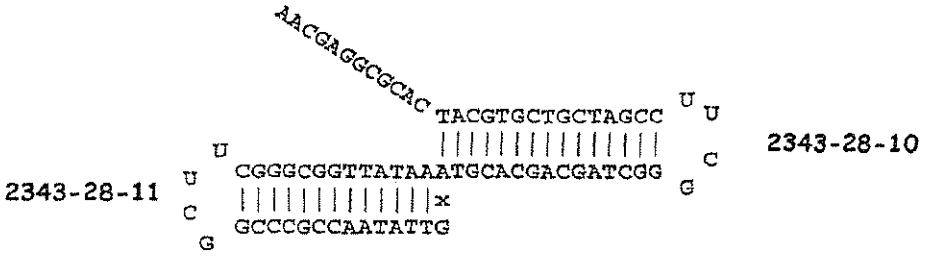
miR-15




| | |
|---|---|
| AACGAGGCGCACATGTGCTGCTAmGmGmCmUmUmCmGmGmCmC | 2343-28-07 |
| mGmGmCmUmUmCmGmGmCmCCACAAACCATTC | 2343-28-08 |
| mUmAmGmCmAmGmCmAmCmAmUmGmUmGmCmGmC | 2343-28-09 |

---

miR-16




| | |
|---|---|
| AACGAGGCGCACTACGTGCTGCTAmGmGmCmUmUmCmGmGmCmC | 2343-28-10 |
| mGmGmCmUmUmCmGmGmCmCCGCCAATATTG | 2343-28-11 |
| mUmAmGmCmAmGmCmAmCmGmUmAmGmUmGmCmGmC | 2343-28-12 |

**FIGURE 32**

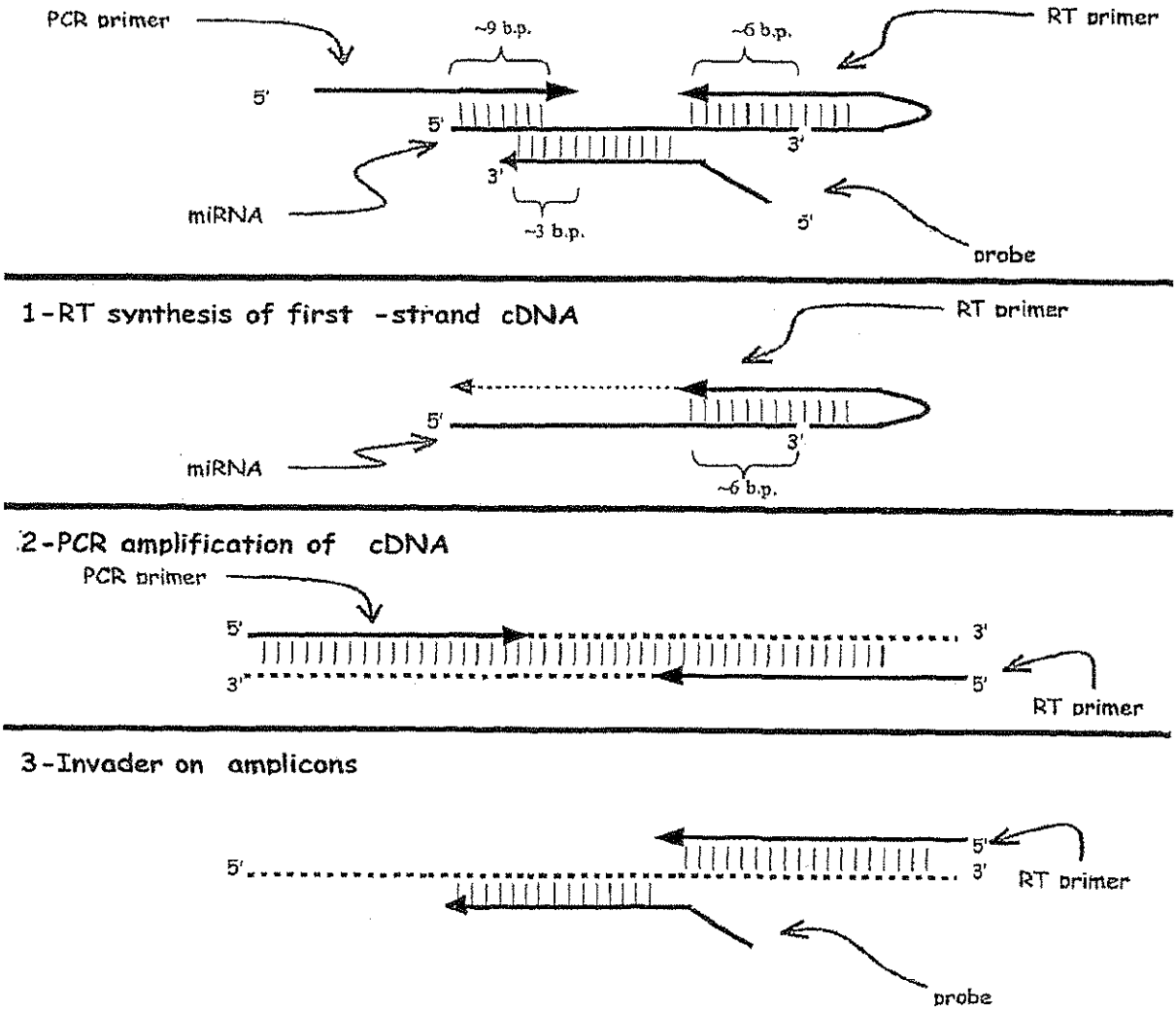

PCR primer
~9 b.p.
~6 b.p.
RT primer
5'
5'
3'
3'
miRNA
~3 b.p.
5'
probe
1-RT synthesis of first -strand cDNA
RT primer
5'
3'
miRNA
~6 b.p.
2-PCR amplification of cDNA
PCR primer
5'
3'
3'
5'
RT primer
3-Invader on amplicons
5'
5'
3'
RT primer
probe

FIGURE 33

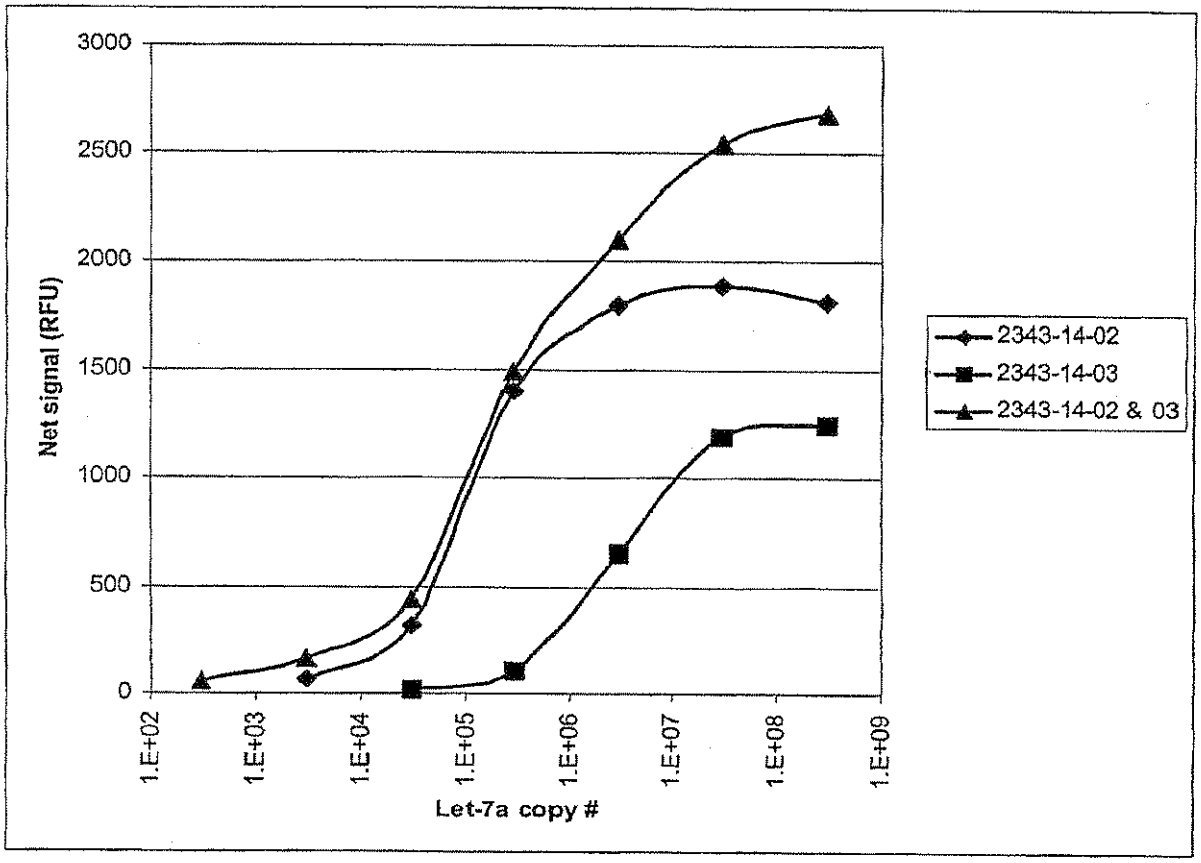
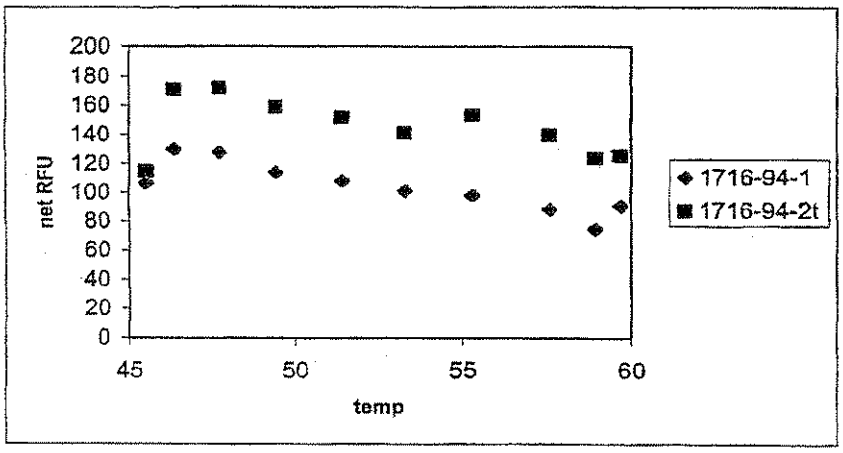

3000
2500
2000
1500
1000
500
0
Net signal (RFU)
1.E+02
1.E+03
1.E+04
1.E+05
1.E+06
1.E+07
1.E+08
1.E+09
Let-7a copy #
2343-14-02
2343-14-03
2343-14-02 & 03

FIGURE 34


200
180
160
140
120
100
80
60
40
20
0
net RFU
45
50
55
60
temp
1716-94-1
1716-94-2t

μ

FIGURE 35

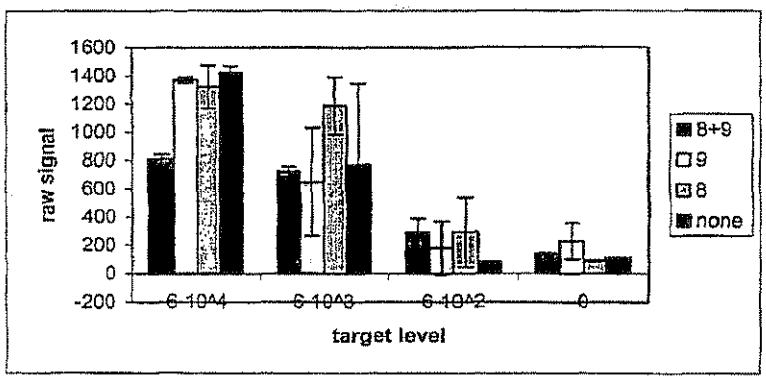

1600
1400
1200
1000
800
600
400
200
0
-200
raw signal
6.10^4
6.10^3
6.10^2
0
target level
8+9
9
8
none

FIGURE 36

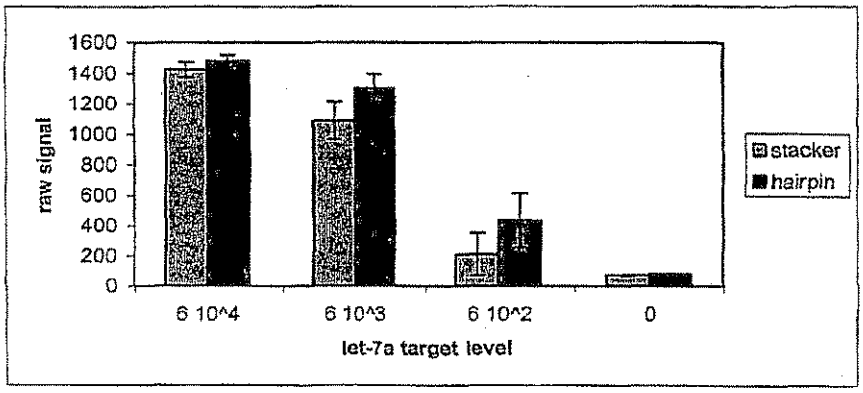

1600
1400
1200
1000
800
600
400
200
0
raw signal
6 10^4
6 10^3
6 10^2
0
let-7a target level
stacker
hairpin

FIGURE 37

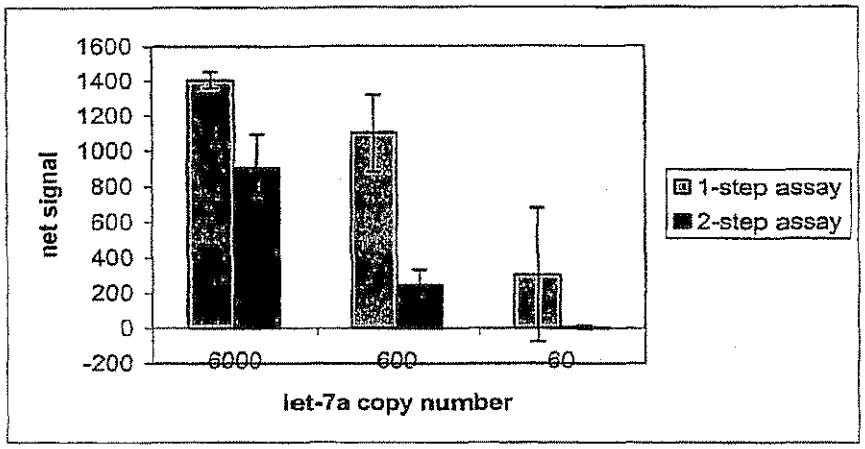

1600
1400
1200
1000
800
600
400
200
0
-200
net signal
6000
600
60
let-7a copy number
1-step assay
2-step assay

**FIGURE 38**

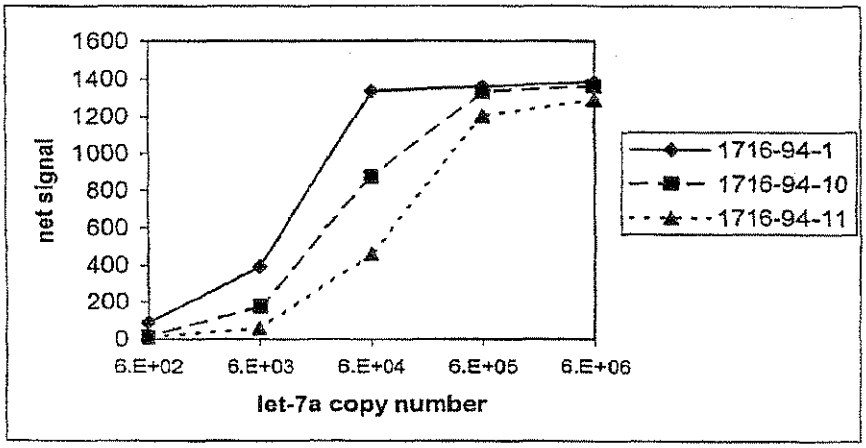

1600
1400
1200
1000
800
600
400
200
0
net signal
6.E+02
6.E+03
6.E+04
6.E+05
6.E+06
let-7a copy number
1716-94-1
1716-94-10
1716-94-11

FIGURE 39

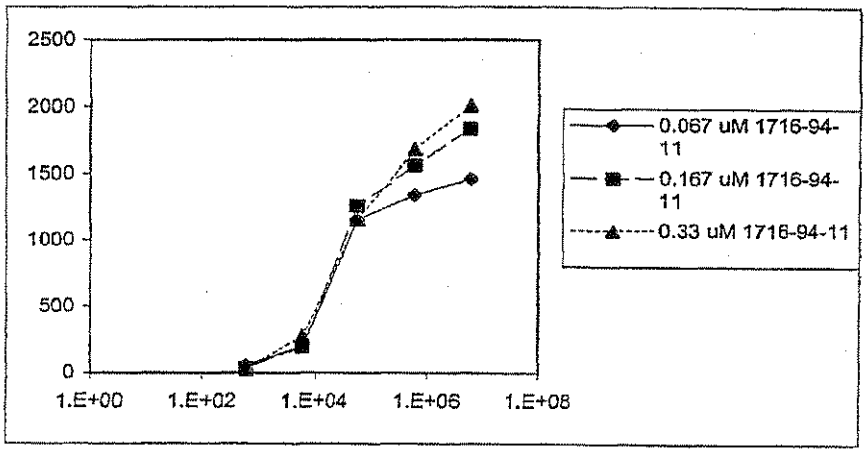
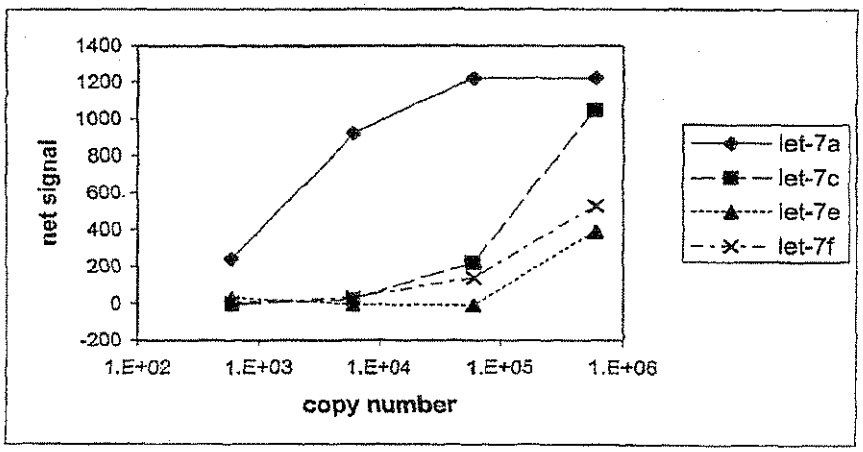
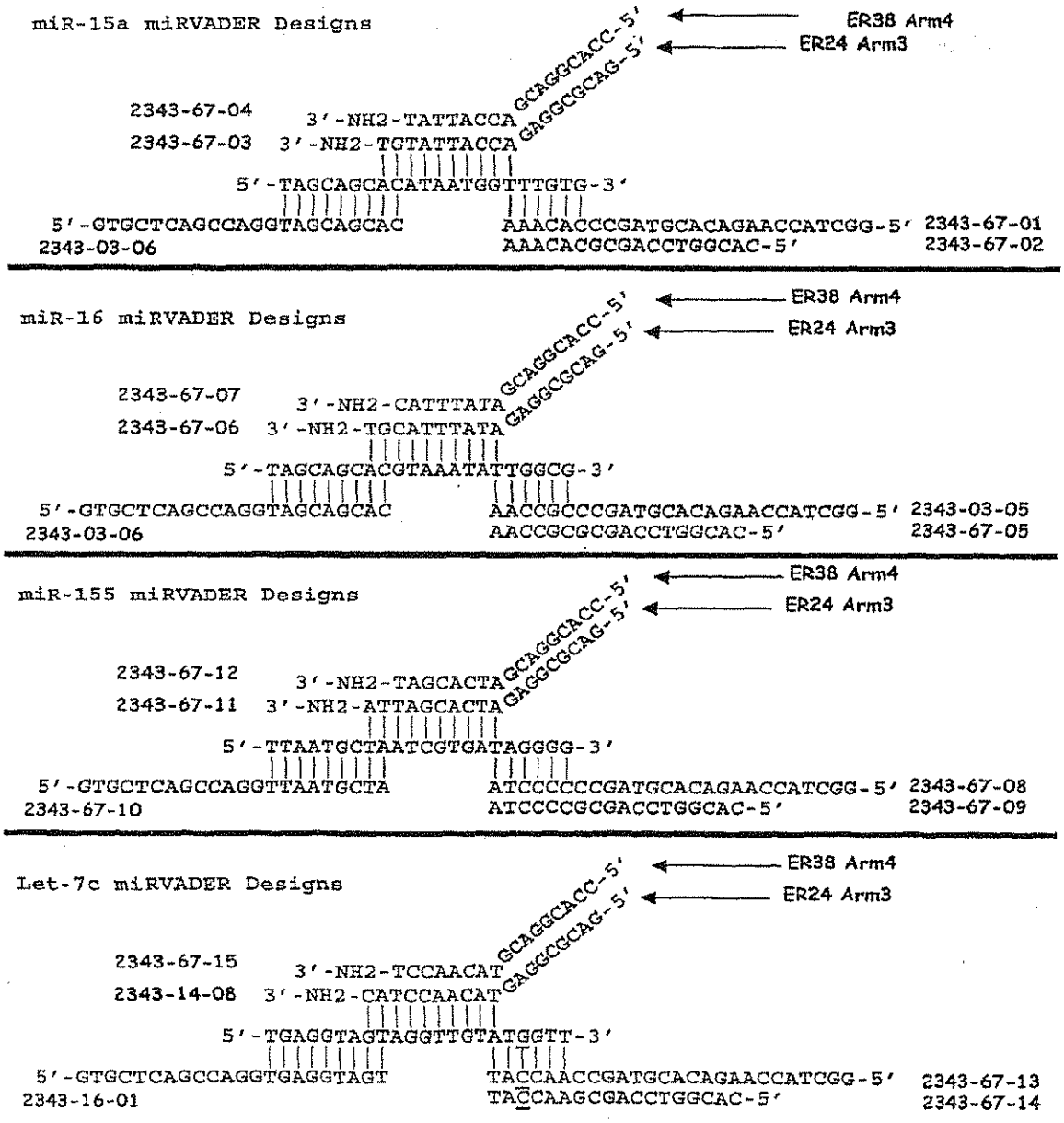

2500
2000
1500
1000
500
0
1.E+00
1.E+02
1.E+04
1.E+06
1.E+08
0.067 uM 1716-94-11
0.167 uM 1716-94-11
0.33 uM 1716-94-11

FIGURE 40


1400
1200
1000
800
600
400
200
0
-200
net signal
1.E+02
1.E+03
1.E+04
1.E+05
1.E+06
copy number
let-7a
let-7c
let-7e
let-7f

## FIGURE 41




Stackers to use with linear RT primer

| | |
|---|---|
| 5'-mCmGmCmTmGmGmAmCmCmGmTmG-3' | 2343-67-34 |
| 5'-mCmGmCmTmGmGmAmCmCmGmT-3' | 2343-67-35 |

**FIGURE 41**
**Continued**

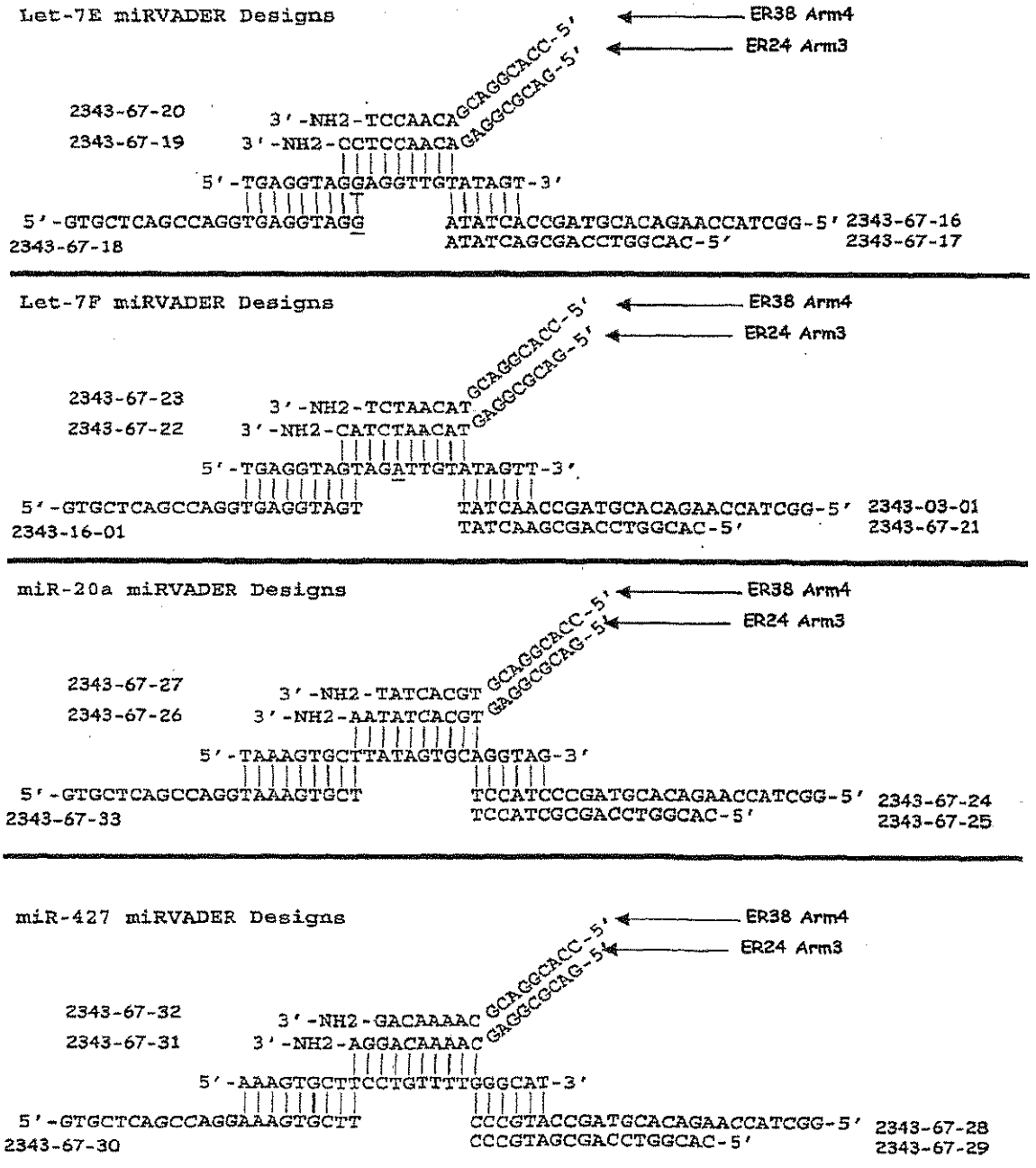


FIGURE 42

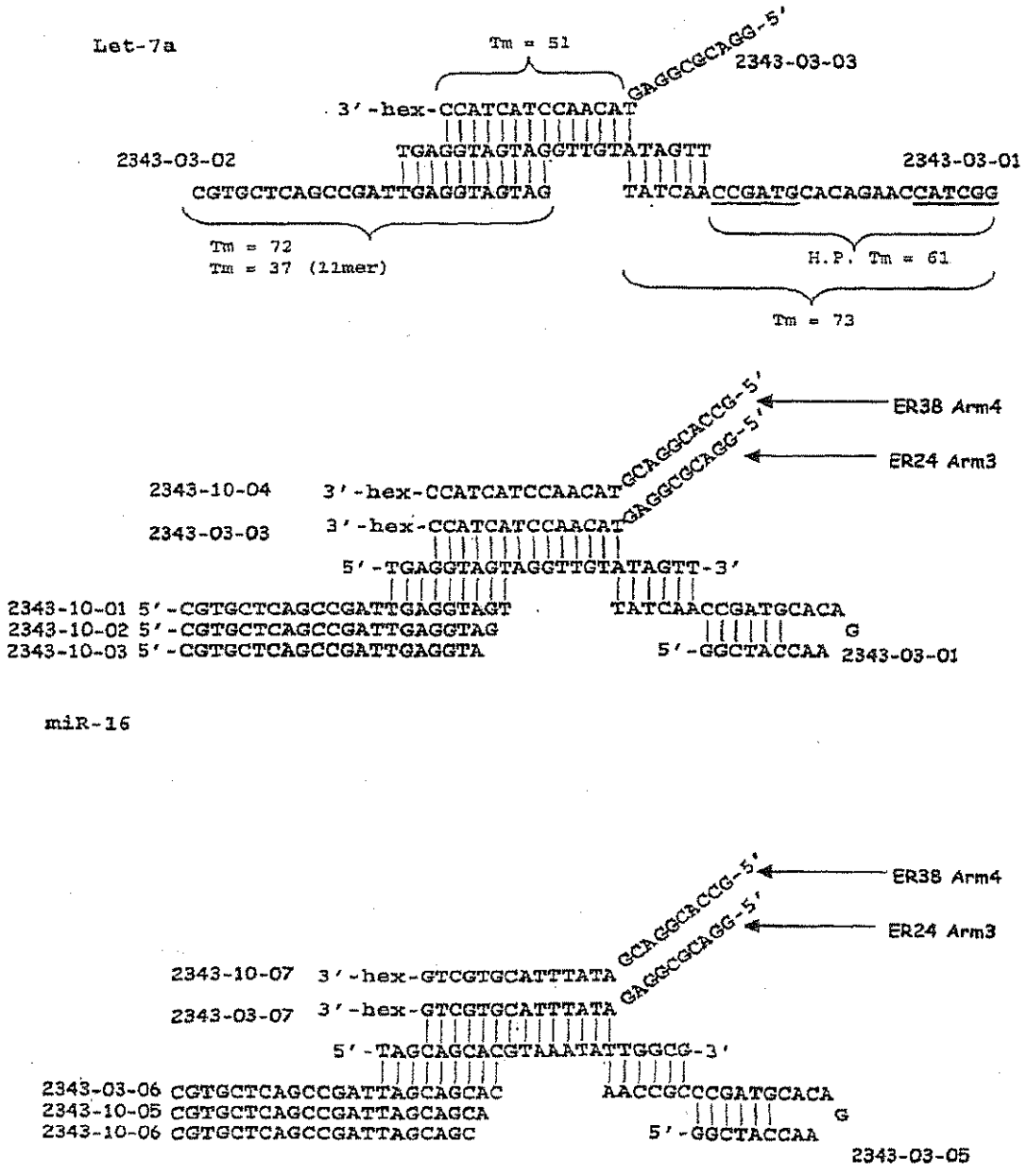
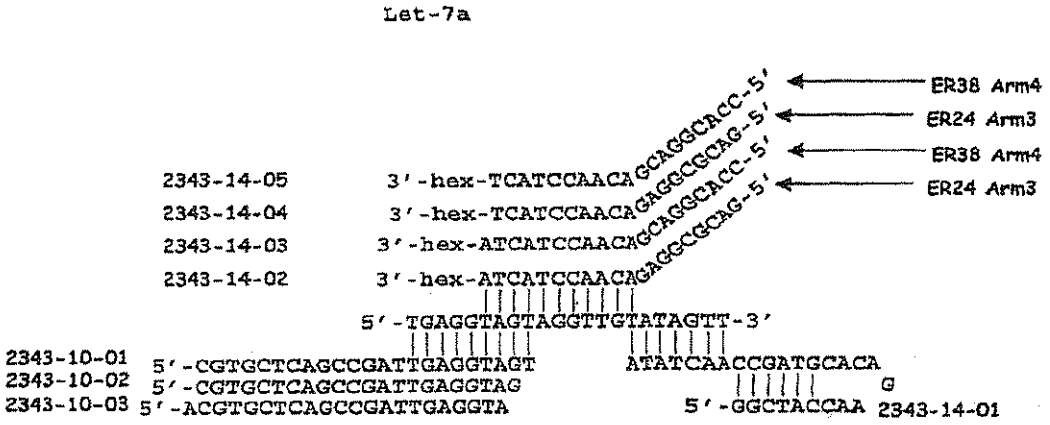
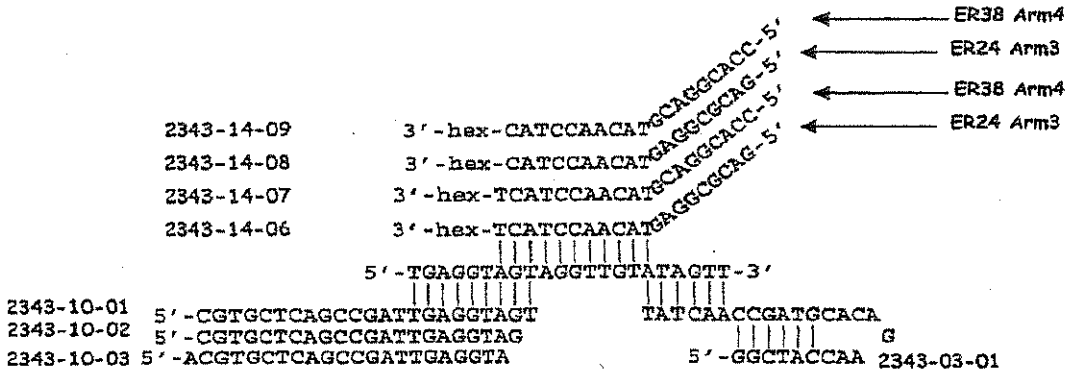
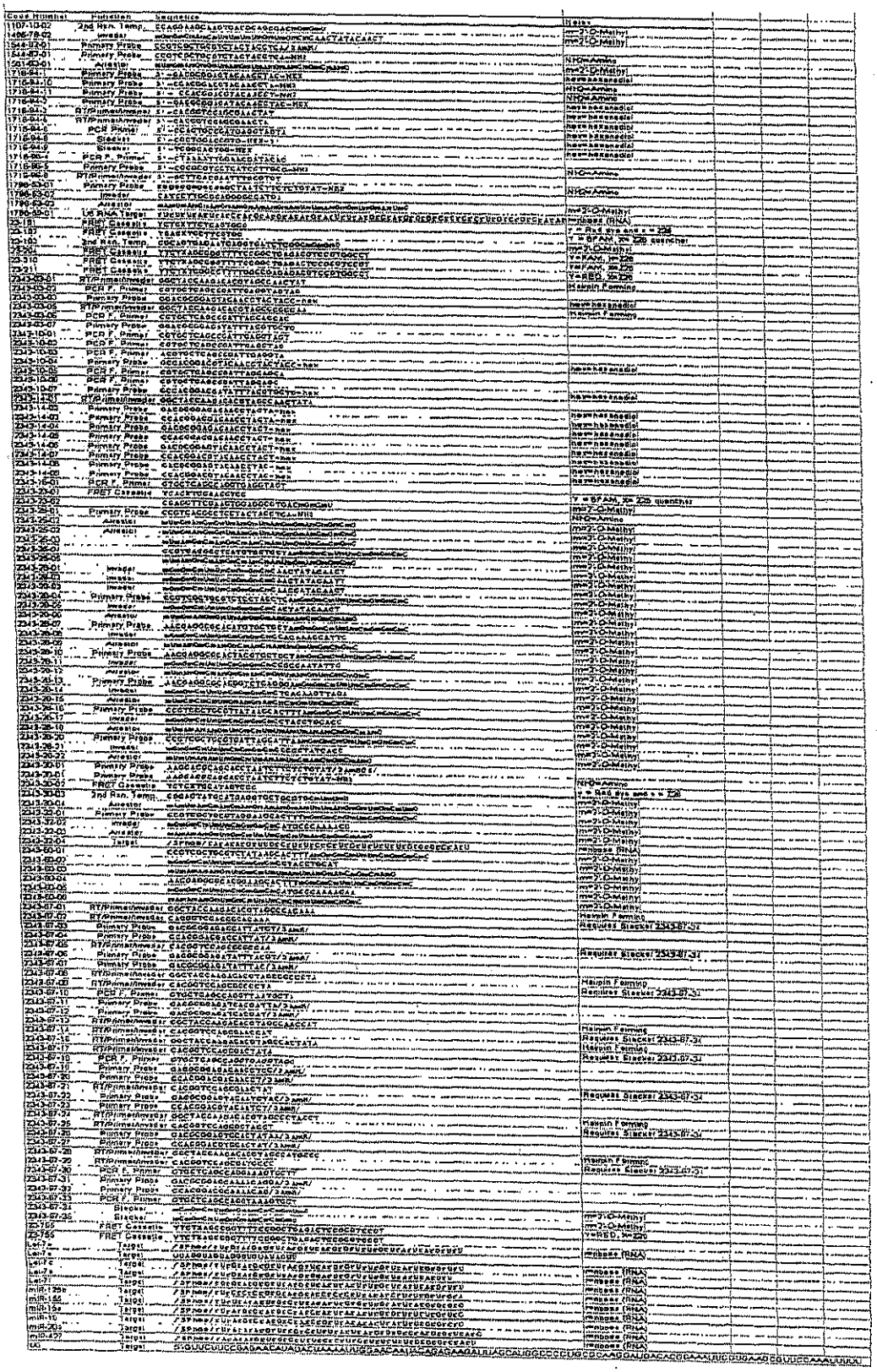

Let-7a
Tm = 51
GAGGCGCAGG-5'
2343-03-03
3'-hex-CCATCATCCAACAT
TGAGGTAGTAGGTTGTATAGTT
2343-03-02
2343-03-01
CGTGCTCAGCCGATTGAGGTAGTAG
TATCAACCGATGCACAGAACCATCGG
Tm = 72
Tm = 37 (11mer)
H.P. Tm = 61
Tm = 73
ER38 Arm4
ER24 Arm3
GCAGGCACCG-5'
GAGGCGCAGG-5'
2343-10-04 3'-hex-CCATCATCCAACAT
2343-03-03 3'-hex-CCATCATCCAACAT
5'-TGAGGTAGTAGGTTGTATAGTT-3'
2343-10-01 5'-CGTGCTCAGCCGATTGAGGTAGT
2343-10-02 5'-CGTGCTCAGCCGATTGAGGTAG
2343-10-03 5'-CGTGCTCAGCCGATTGAGGTA
TATCAACCGATGCACA
G
5'-GGCTACCAA
2343-03-01
miR-16
ER38 Arm4
ER24 Arm3
GCAGGCACCG-5'
GAGGCGCAGG-5'
2343-10-07 3'-hex-GTCGTGCATTTATA
2343-03-07 3'-hex-GTCGTGCATTTATA
5'-TAGCAGCACGTAAATATTGGCG-3'
2343-03-06 CGTGCTCAGCCGATTAGCAGCAC
2343-10-05 CGTGCTCAGCCGATTAGCAGCA
2343-10-06 CGTGCTCAGCCGATTAGCAGC
AACCGCCCGATGCACA
G
5'-GGCTACCAA
2343-03-05

**FIGURE 42**
**Continued**




1- 7 base RT primer
2- 11 mer and 10mer probes with Arms 3 &4
3- 4, 3, and 2 base overlap with PCR primer




1- 6 base RT primer
2- 11 mer and 10mer probes with Arms 3 &4
3- 3, 2, and 1 base overlap with PCR primer

# FIGURE 43