FIGURE 1A

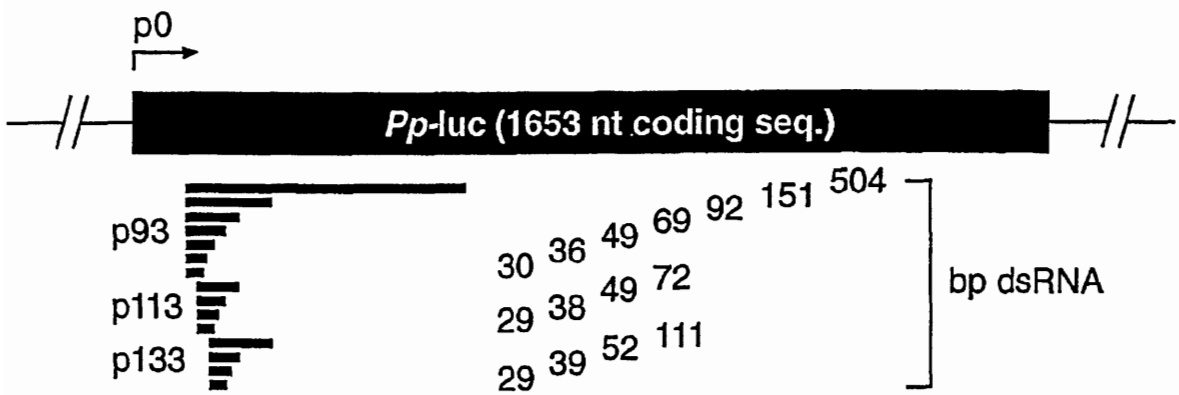


FIGURE 1B

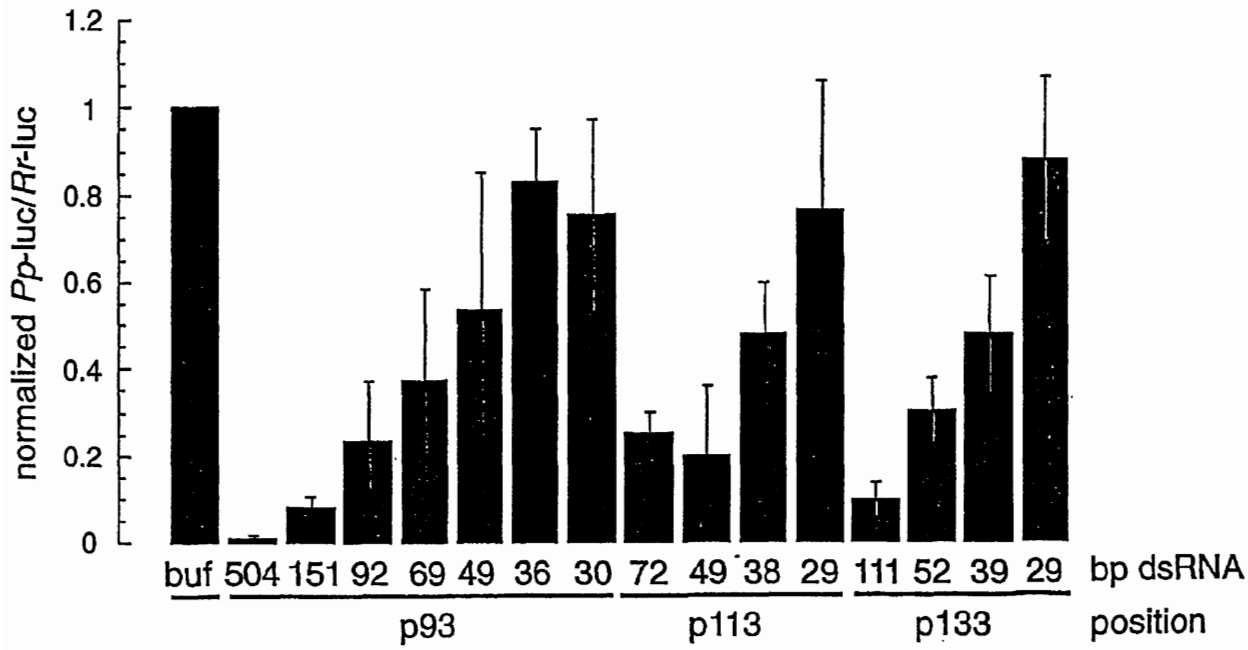


FIGURE 2

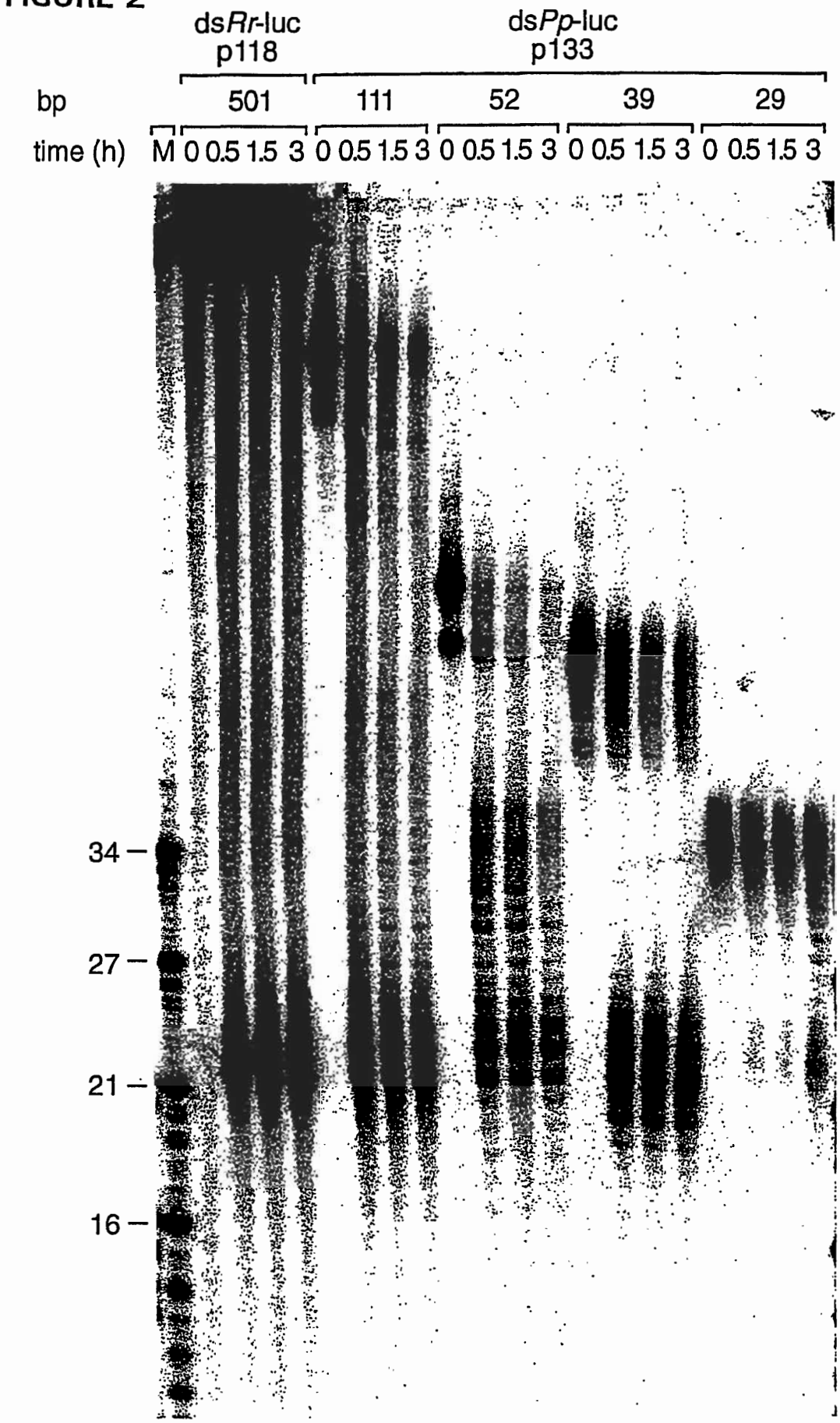

dsRr-luc
p118
dsPp-luc
p133
bp
501
111
52
39
29
time (h)
M 0 0.5 1.5 3 0 0.5 1.5 3 0 0.5 1.5 3 0 0.5 1.5 3 0 0.5 1.5 3
34
27
21
16

Figure 3A

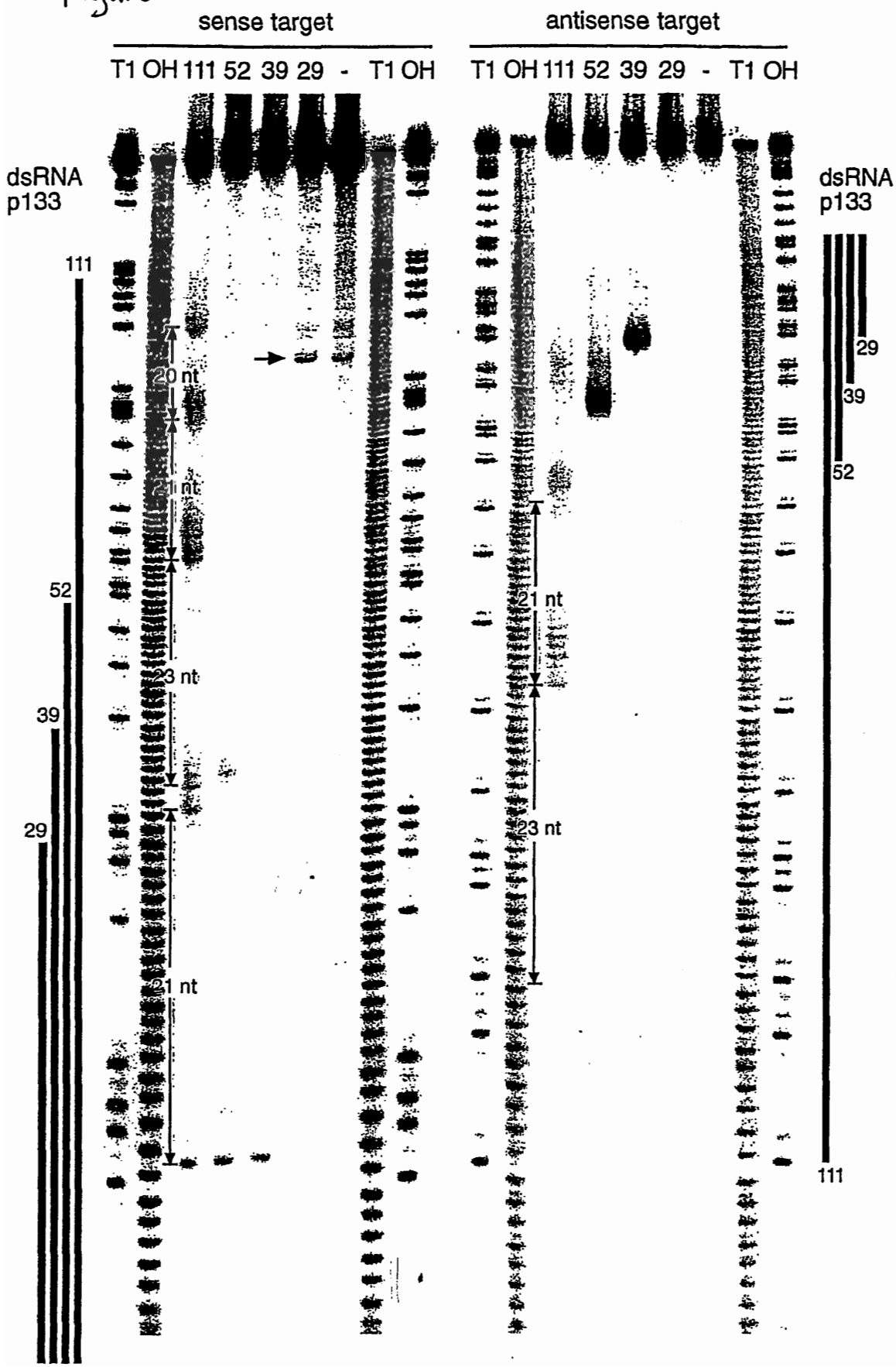

sense target
antisense target
T1 OH 111 52 39 29 - T1 OH
T1 OH 111 52 39 29 - T1 OH
dsRNA
p133
dsRNA
p133
111
52
39
29
20 nt
21 nt
23 nt
21 nt
21 nt
23 nt
29
39
52
111

FIGURE 3B

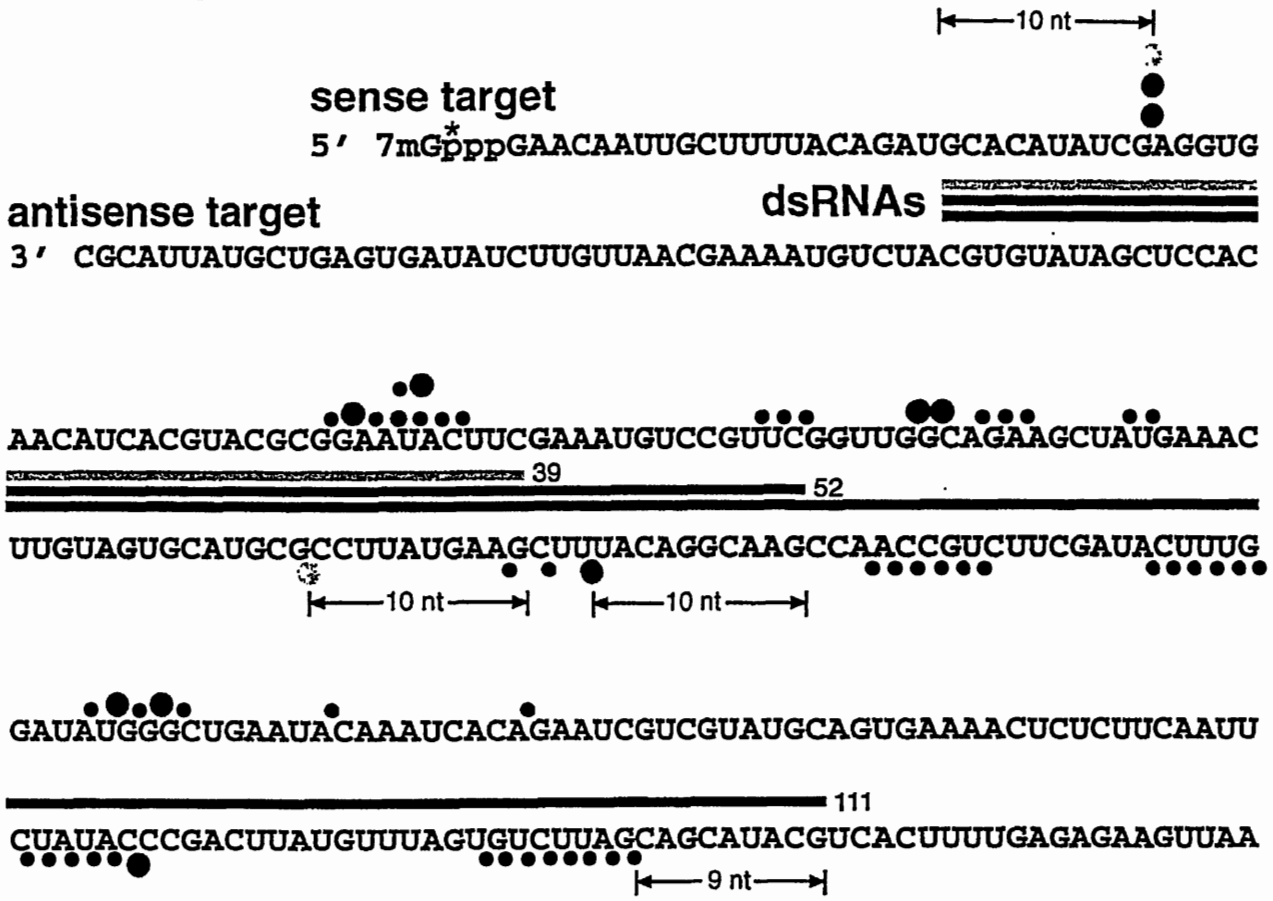
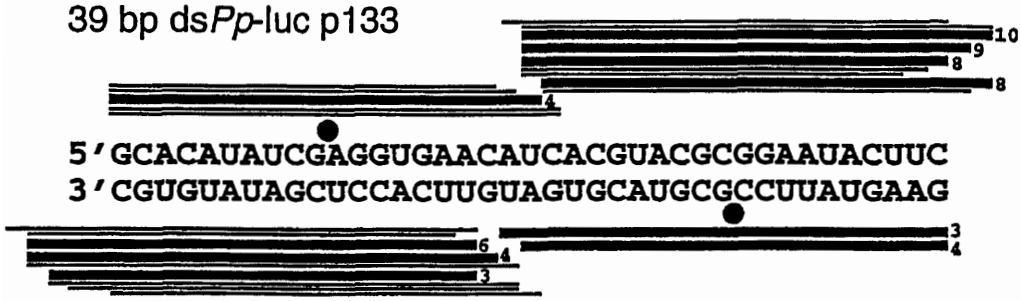
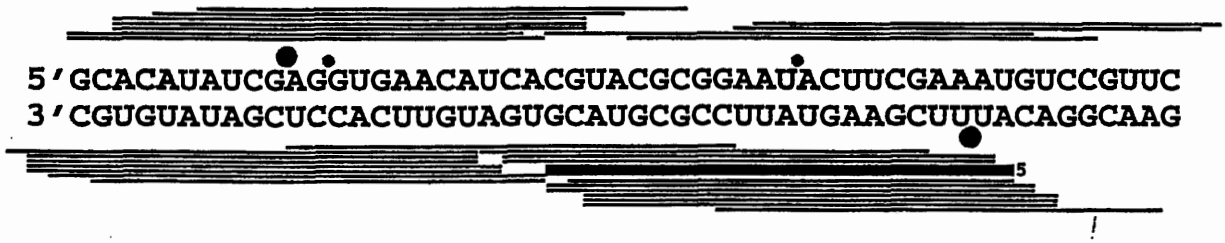

10 nt
sense target
5′ 7mG*pppGAACAAUUGCUUUUACAGAUGCACAUAUCGAGGUG
antisense target
dsRNAs
3′ CGCAUUAUGCUGAGUGAUAUCUUGUUAACGAAAAUGUCUACGUGUAUAGCUCCAC
AACAUCACGUACGCGGAAUACUUCGAAAUGUCCGUUCGGUUGGCAGAAGCUAUGAAAC
39
52
UUGUAGUGCAUGCGCCUUAUGAAGCUUUACAGGCAAGCCAACCGUCUUCGAUACUUUG
10 nt
10 nt
GAUAUGGGCUGAAUACAAAUCACAGAAUCGUCGUAUGCAGUGAAAACUCUCUUCAAUU
111
CUAUACCCGACUUAUGUUUAGUGUCUUAGCAGCAUACGUCACUUUUGAGAGAAGUUAA
9 nt
CUUUAUGCCUAUAGUGUCACCUAAAU 3′
GAAAUACGGppp*Gm7 5′

FIGURE 4A

39 bp ds*Pp*-luc p133



52 bp ds*Pp*-luc p133



111 bp ds*Pp*-luc p133

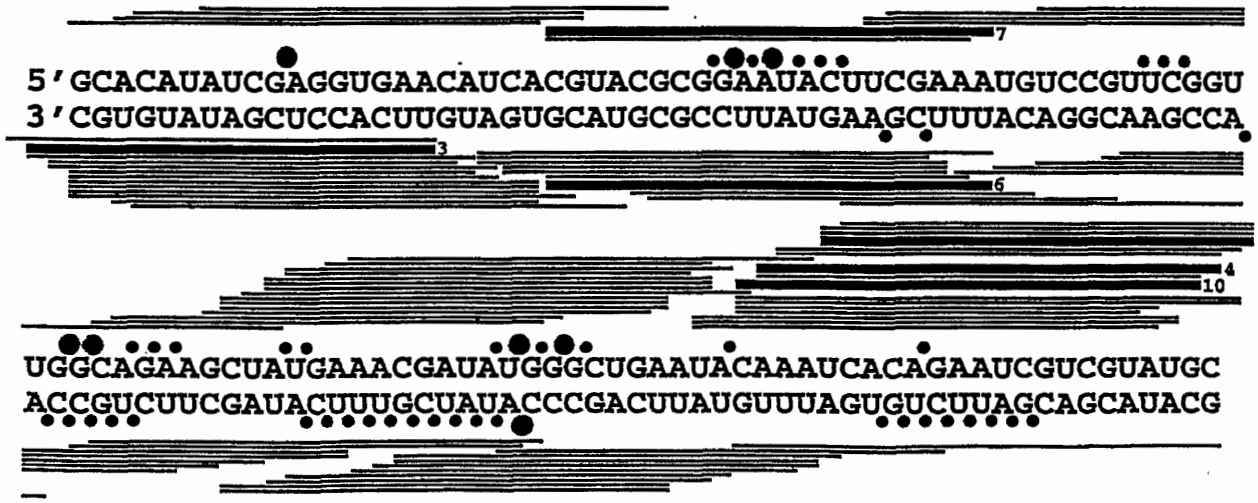


FIGURE 4B

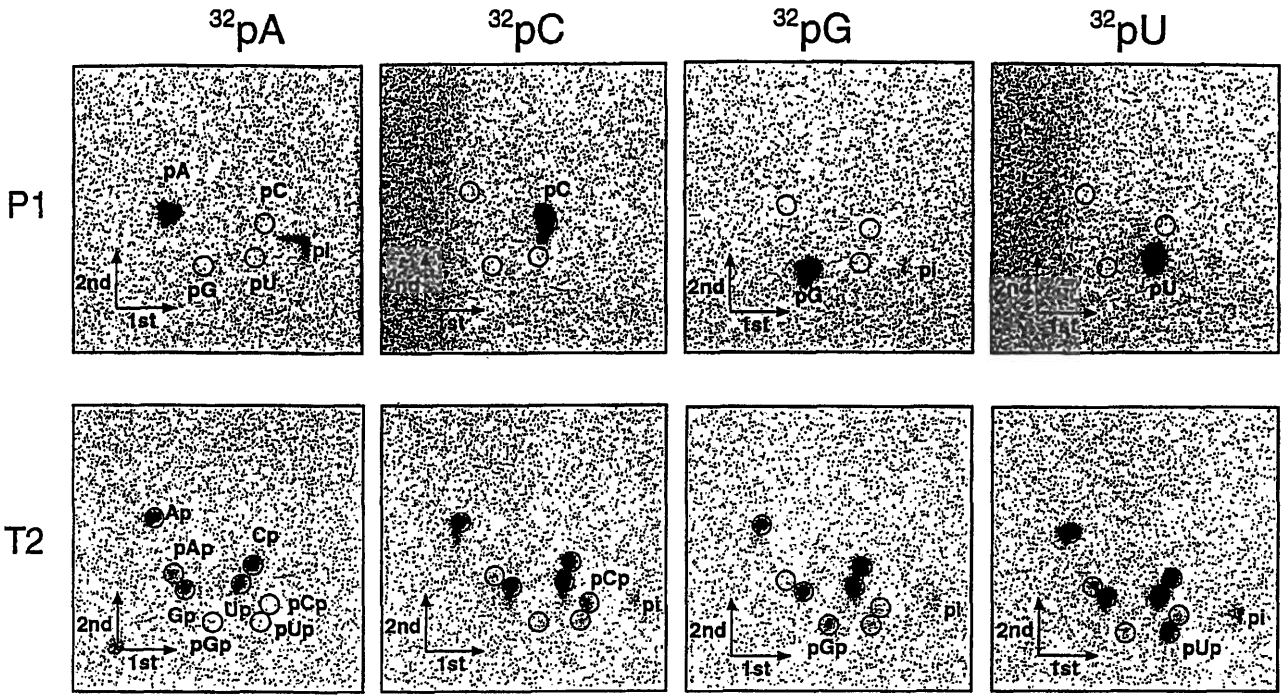

32pA
32pC
32pG
32pU
P1
T2
pA
pC
pG
pU
pi
Ap
Cp
pAp
Gp
Up
pCp
pUp
pGp
2nd
1st

Figure 5A

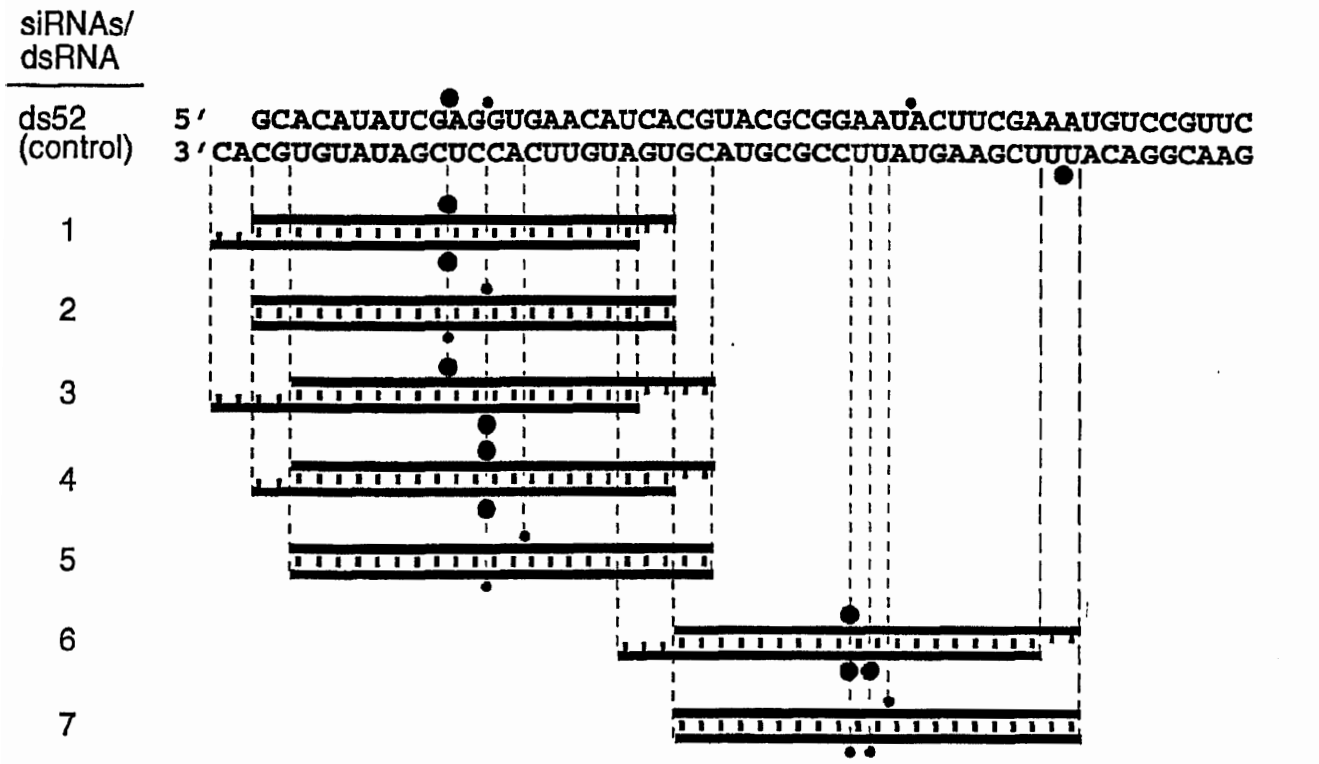

siRNAs/
dsRNA
ds52
(control)
5′ GCACAUAUCGAGGUGAACAUCACGUACGCGGAAUACUUCGAAAUGUCCGUUC
3′ CACGUGUAUAGCUCCACUUGUAGUGCAUGCGCCUUAUGAAGCUUUACAGGCAAG
1
2
3
4
5
6
7

FIGURE 5B

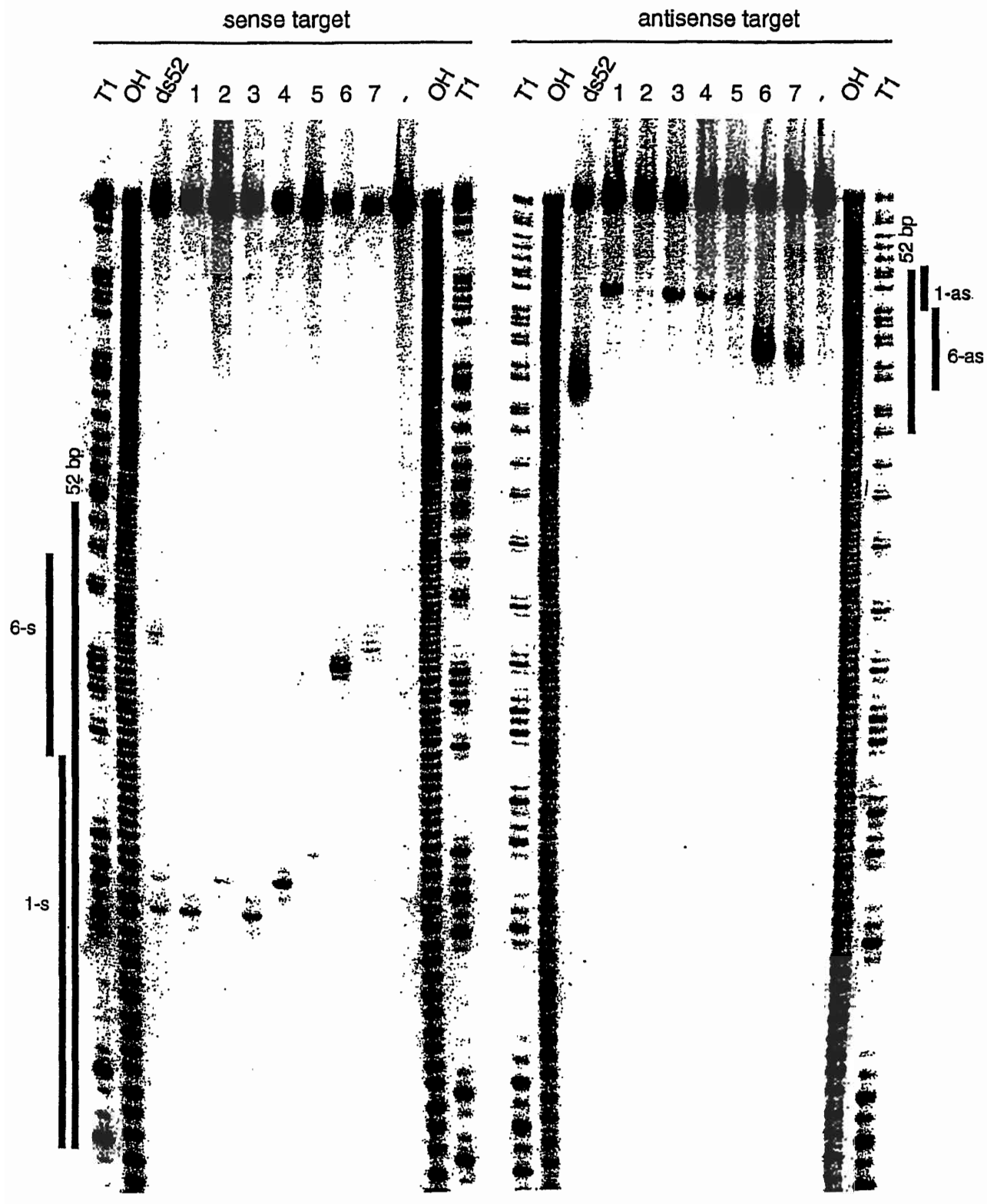

sense target
antisense target
T1
OH
ds52
1
2
3
4
5
6
7
OH
T1
52 bp
6-s
1-s
52 bp
1-as
6-as

FIGURE 6A

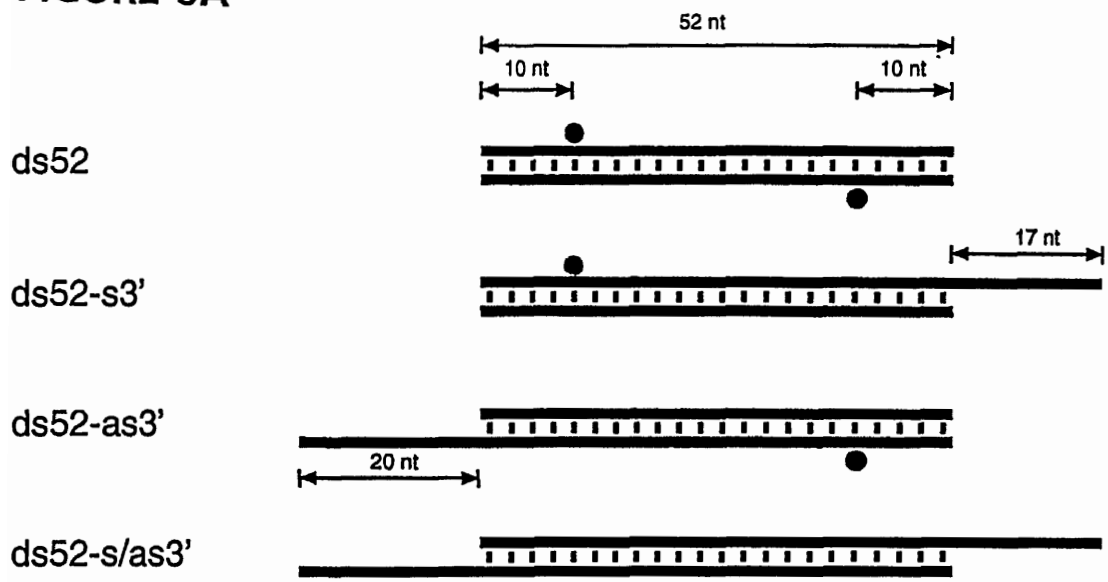
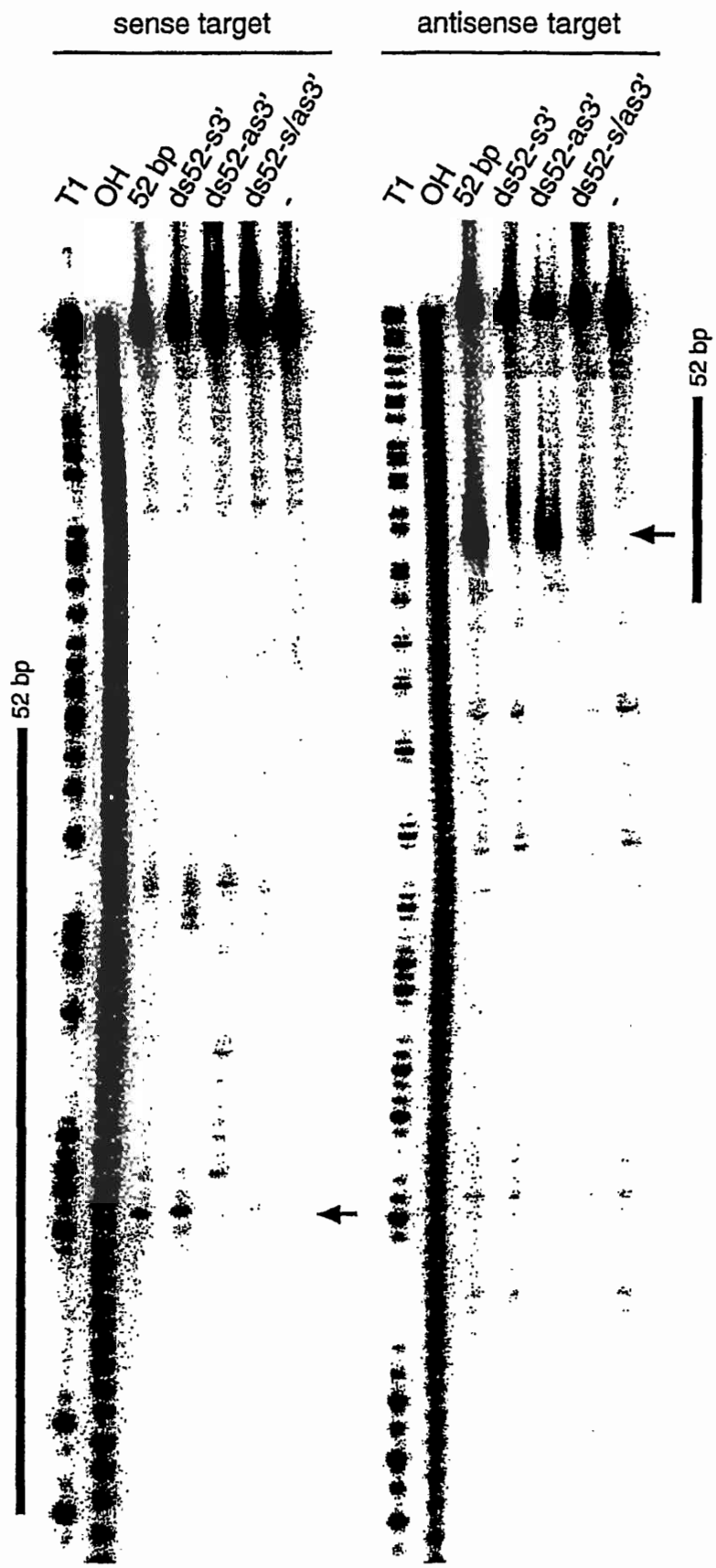


FIGURE 6B


sense target
antisense target
T1
OH
52 bp
ds52-s3'
ds52-as3'
ds52-s/as3'
-
52 bp
52 bp

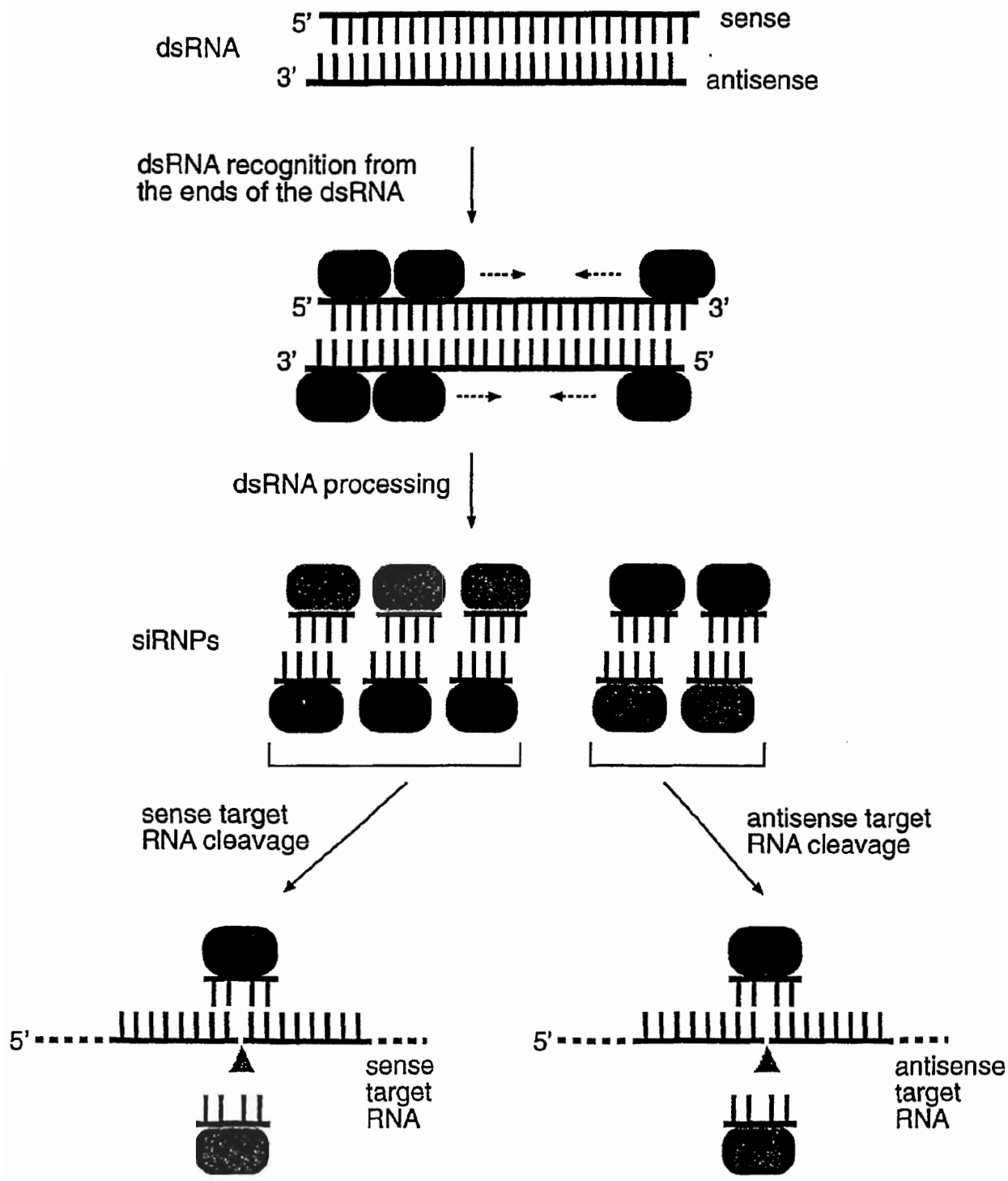
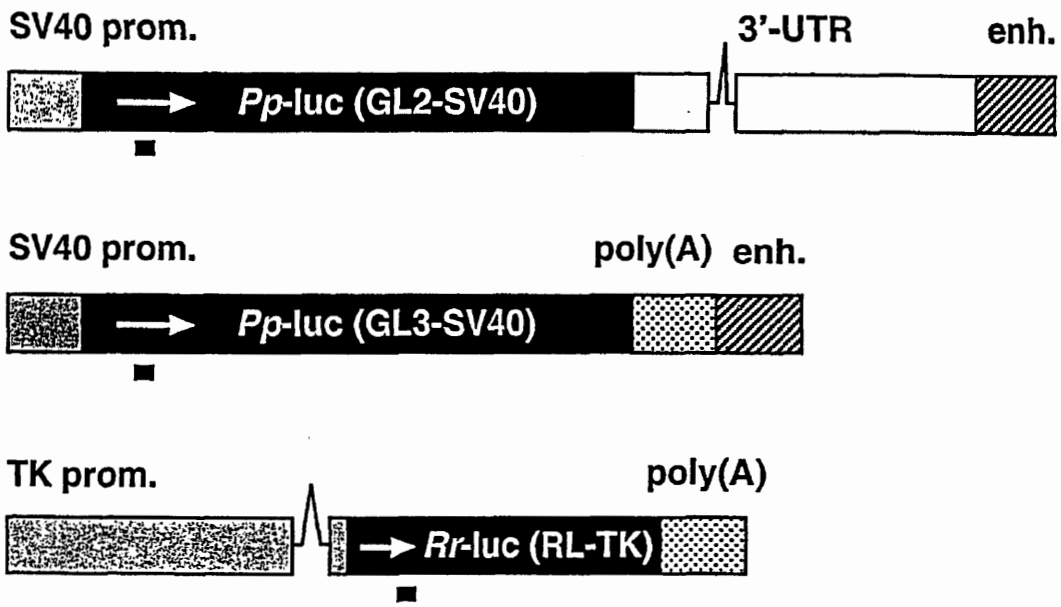

dsRNA
5'
3'
sense
antisense
dsRNA recognition from the ends of the dsRNA
5'
3'
3'
5'
dsRNA processing
siRNPs
sense target RNA cleavage
antisense target RNA cleavage
5'
sense target RNA
5'
antisense target RNA

Figure 7

a



b

siRNA duplex	
uGL2	5′ CGUACGCGGAAUACUUCGAUU UUGCAUGCGCCUUAUGAAGCU 5′
GL2	5′ CGUACGCGGAAUACUUCGATT TTGCAUGCGCCUUAUGAAGCU 5′
GL3	5′ CUUACGCUGAGUACUUCGATT TTGAAUGCGACUCAUGAAGCU 5′
invGL2	5′ AGCUUCAUAAGGCGCAUGCTT TTUCGAAGUAUUCCGCGUACG 5′
RL	5′ AAACAUGCAGAAAAUGCUGTT TTUUUGUACGUCUUUUACGAC 5′

Figure 8

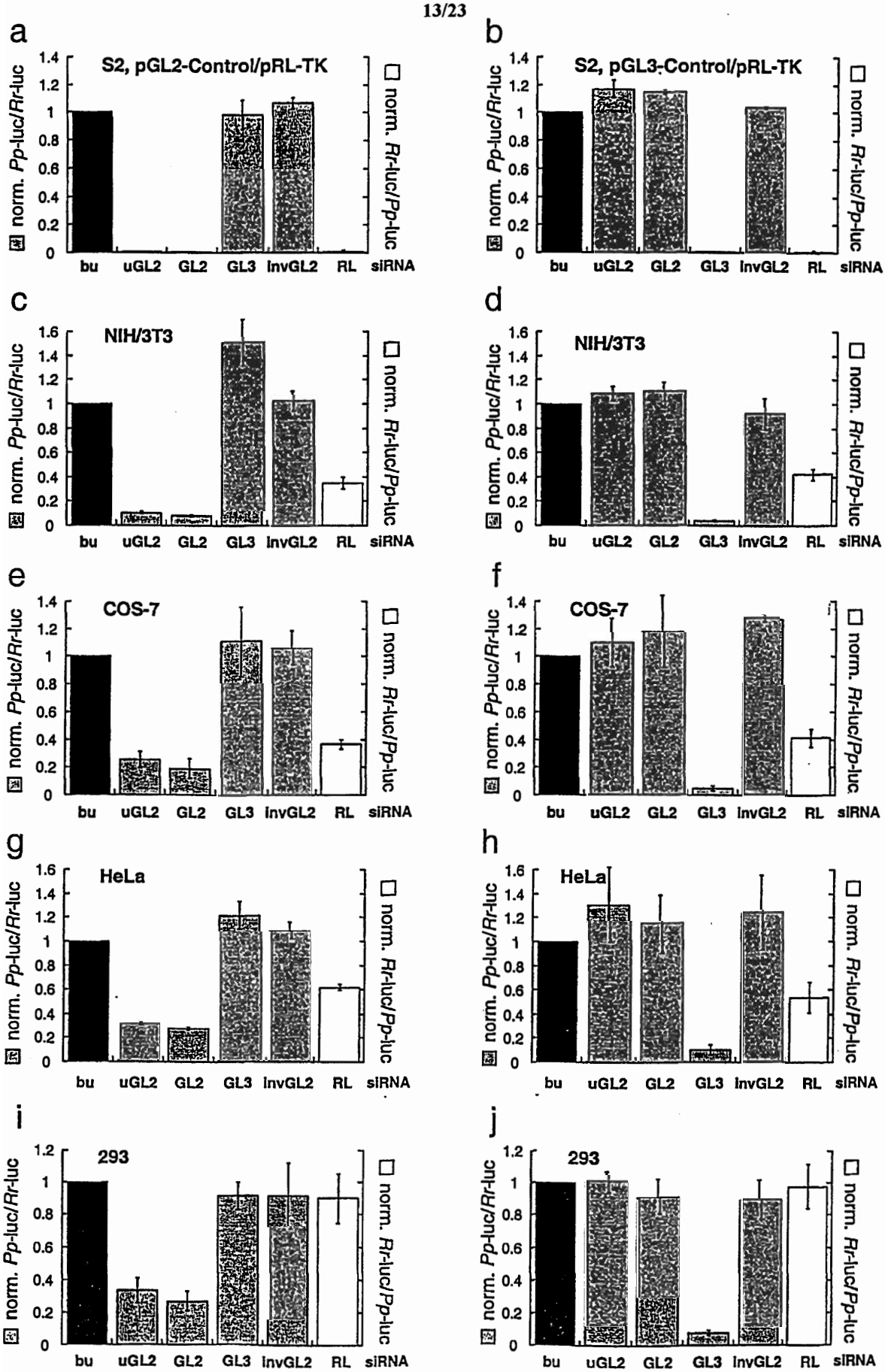

a
S2, pGL2-Control/pRL-TK
b
S2, pGL3-Control/pRL-TK
c
NIH/3T3
d
NIH/3T3
e
COS-7
f
COS-7
g
HeLa
h
HeLa
i
293
j
293
norm. Pp-luc/Rr-luc
norm. Rr-luc/Pp-luc
bu
uGL2
GL2
GL3
invGL2
RL
siRNA

Figure 9

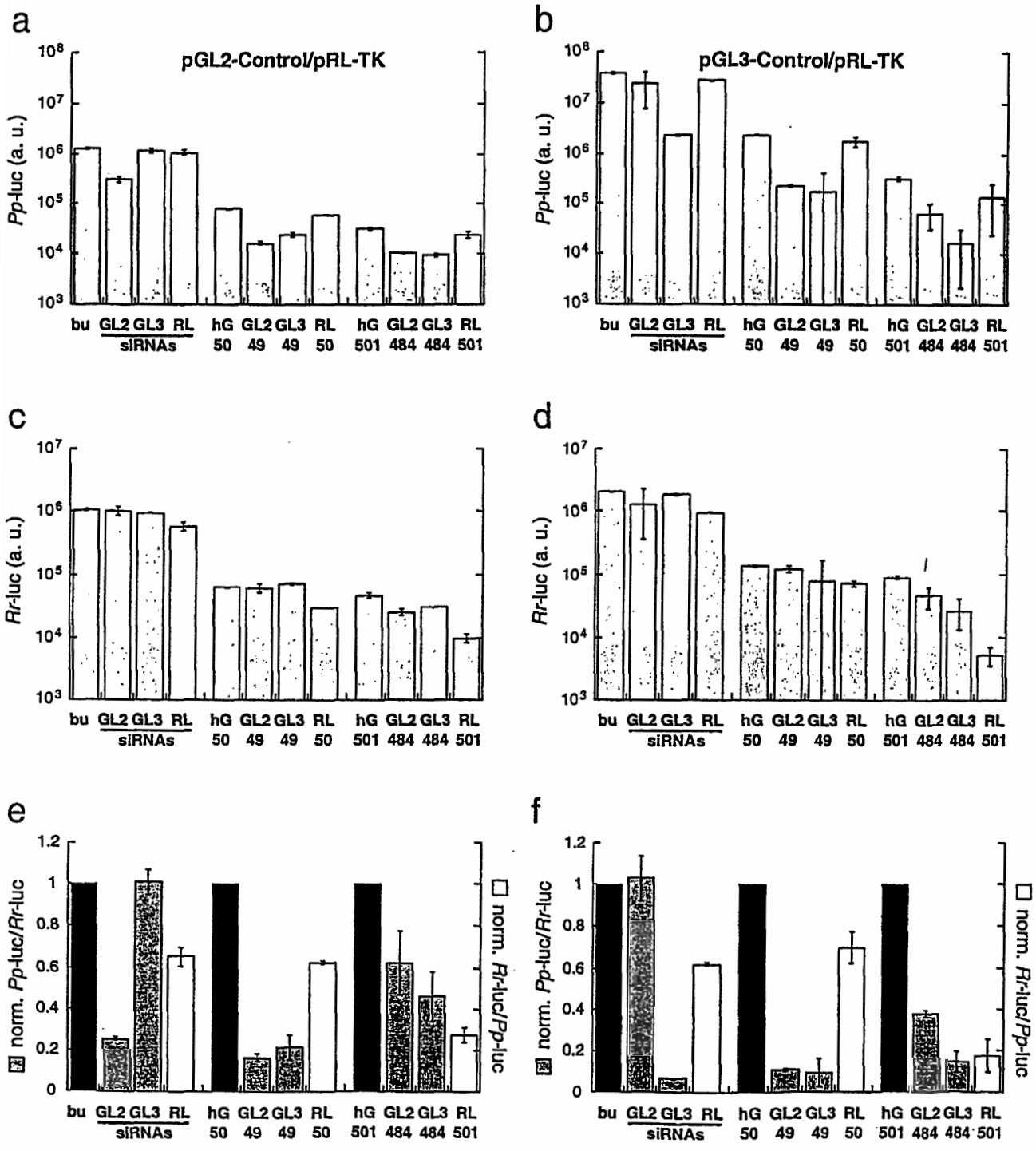
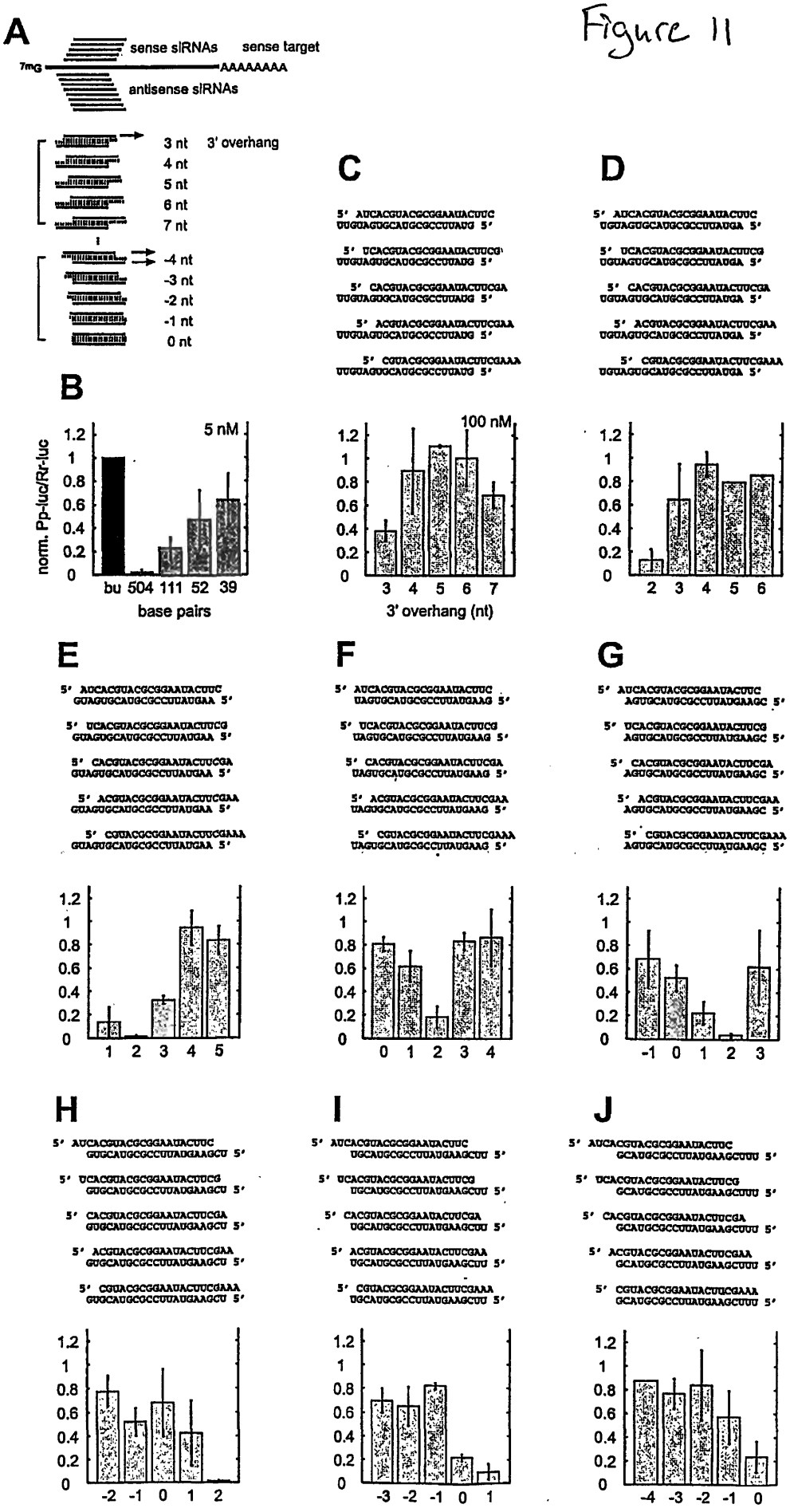

a
pGL2-Control/pRL-TK
Pp-luc (a. u.)
b
pGL3-Control/pRL-TK
Pp-luc (a. u.)
c
Rr-luc (a. u.)
d
Rr-luc (a. u.)
e
norm. Pp-luc/Rr-luc
norm. Rr-luc/Pp-luc
f
norm. Pp-luc/Rr-luc
norm. Rr-luc/Pp-luc
bu GL2 GL3 RL hG GL2 GL3 RL hG GL2 GL3 RL
siRNAs
50 49 49 50 501 484 484 501

Figure 10

Figure 11


A
sense siRNAs
sense target
7mG
AAAAAAAA
antisense siRNAs
3 nt
3' overhang
4 nt
5 nt
6 nt
7 nt
-4 nt
-3 nt
-2 nt
-1 nt
0 nt
B
5 nM
norm. Pp-luc/Rr-luc
bu 504 111 52 39
base pairs
C
100 nM
3' overhang (nt)
D
E
F
G
H
I
J

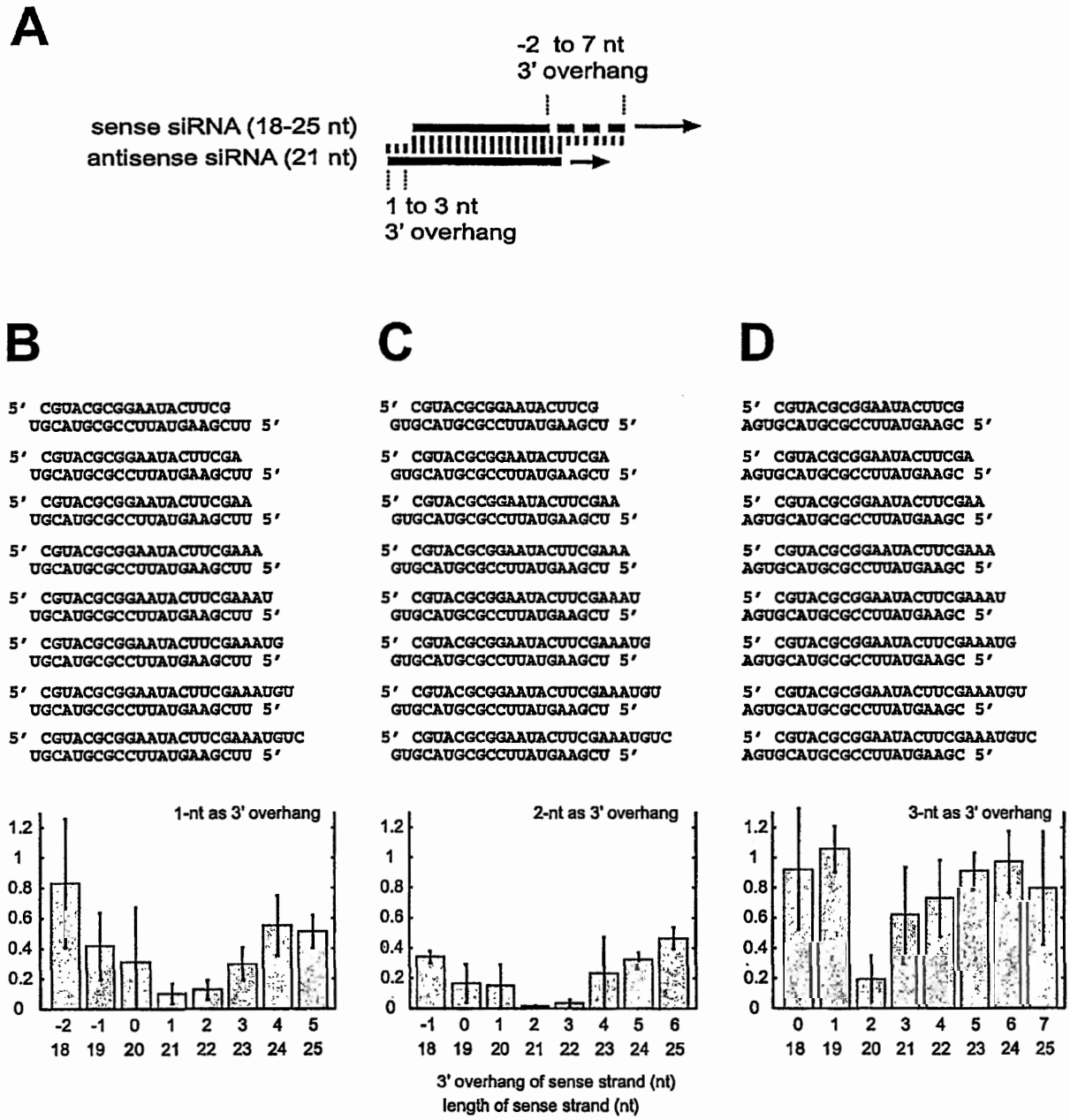

A
-2 to 7 nt
3' overhang
sense siRNA (18-25 nt)
antisense siRNA (21 nt)
1 to 3 nt
3' overhang
B
5' CGUACGCGGAAUACUUCG
UGCAUGCGCCUUAUGAAGCUU 5'
5' CGUACGCGGAAUACUUCGA
UGCAUGCGCCUUAUGAAGCUU 5'
5' CGUACGCGGAAUACUUCGAA
UGCAUGCGCCUUAUGAAGCUU 5'
5' CGUACGCGGAAUACUUCGAAA
UGCAUGCGCCUUAUGAAGCUU 5'
5' CGUACGCGGAAUACUUCGAAAU
UGCAUGCGCCUUAUGAAGCUU 5'
5' CGUACGCGGAAUACUUCGAAAUG
UGCAUGCGCCUUAUGAAGCUU 5'
5' CGUACGCGGAAUACUUCGAAAUGU
UGCAUGCGCCUUAUGAAGCUU 5'
5' CGUACGCGGAAUACUUCGAAAUGUC
UGCAUGCGCCUUAUGAAGCUU 5'
C
5' CGUACGCGGAAUACUUCG
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGA
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAAU
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAAUG
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAAUGU
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAAUGUC
GUGCAUGCGCCUUAUGAAGCU 5'
D
5' CGUACGCGGAAUACUUCG
AGUGCAUGCGCCUUAUGAAGC 5'
5' CGUACGCGGAAUACUUCGA
AGUGCAUGCGCCUUAUGAAGC 5'
5' CGUACGCGGAAUACUUCGAA
AGUGCAUGCGCCUUAUGAAGC 5'
5' CGUACGCGGAAUACUUCGAAA
AGUGCAUGCGCCUUAUGAAGC 5'
5' CGUACGCGGAAUACUUCGAAAU
AGUGCAUGCGCCUUAUGAAGC 5'
5' CGUACGCGGAAUACUUCGAAAUG
AGUGCAUGCGCCUUAUGAAGC 5'
5' CGUACGCGGAAUACUUCGAAAUGU
AGUGCAUGCGCCUUAUGAAGC 5'
5' CGUACGCGGAAUACUUCGAAAUGUC
AGUGCAUGCGCCUUAUGAAGC 5'
1-nt as 3' overhang
2-nt as 3' overhang
3-nt as 3' overhang
norm. Pp-luc/Rr-luc
1.2
1
0.8
0.6
0.4
0.2
0
-2 -1 0 1 2 3 4 5
18 19 20 21 22 23 24 25
-1 0 1 2 3 4 5 6
18 19 20 21 22 23 24 25
0 1 2 3 4 5 6 7
18 19 20 21 22 23 24 25
3' overhang of sense strand (nt)
length of sense strand (nt)

Fig. 13

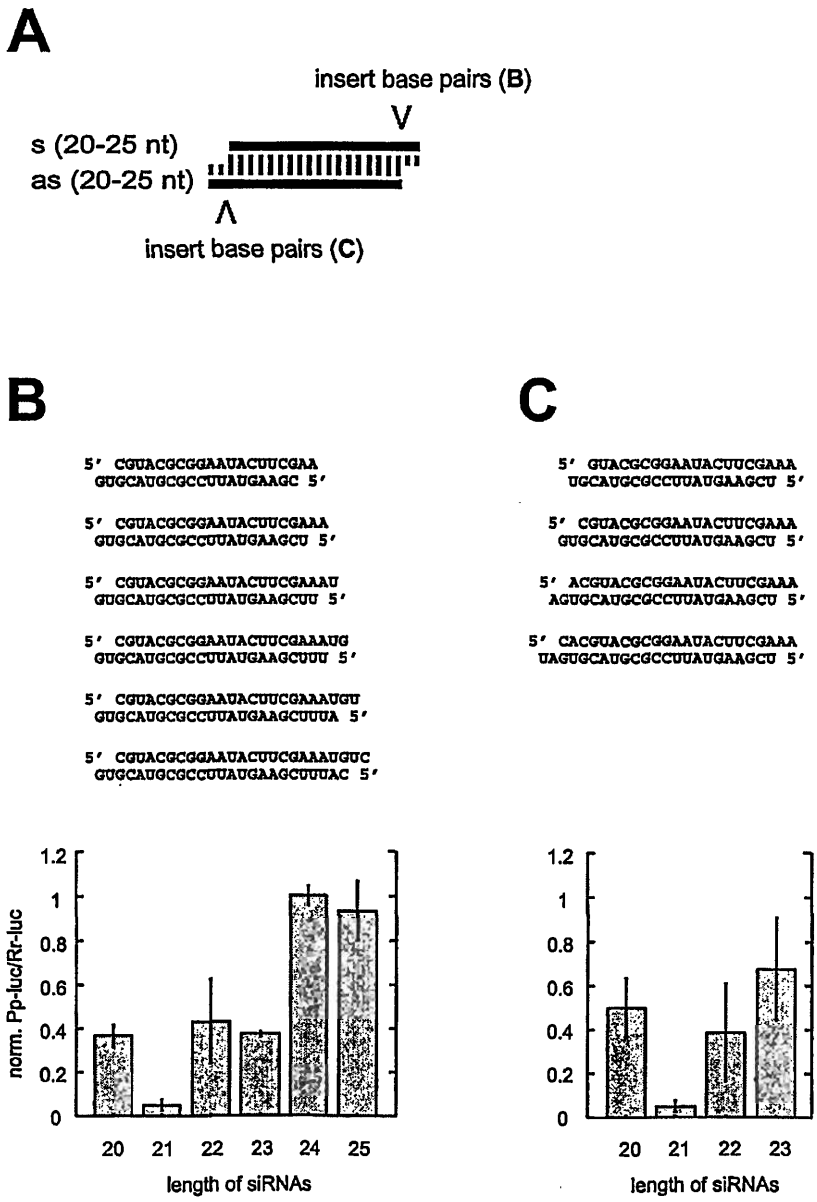

A
insert base pairs (B)
s (20-25 nt)
as (20-25 nt)
insert base pairs (C)
B
5' CGUACGCGGAAUACUUCGAA
GUGCAUGCGCCUUAUGAAGC 5'
5' CGUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAAU
GUGCAUGCGCCUUAUGAAGCUU 5'
5' CGUACGCGGAAUACUUCGAAAUG
GUGCAUGCGCCUUAUGAAGCUUU 5'
5' CGUACGCGGAAUACUUCGAAAUGU
GUGCAUGCGCCUUAUGAAGCUUUA 5'
5' CGUACGCGGAAUACUUCGAAAUGUC
GUGCAUGCGCCUUAUGAAGCUUUAC 5'
C
5' GUACGCGGAAUACUUCGAAA
UGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' ACGUACGCGGAAUACUUCGAAA
AGUGCAUGCGCCUUAUGAAGCU 5'
5' CACGUACGCGGAAUACUUCGAAA
UAGUGCAUGCGCCUUAUGAAGCU 5'
norm. Pp-luc/Rr-luc
1.2
1
0.8
0.6
0.4
0.2
0
20 21 22 23 24 25
length of siRNAs
1.2
1
0.8
0.6
0.4
0.2
0
20 21 22 23
length of siRNAs

Fig. 14

s 5′ CGUACGCGGAAUACUUCGAAA
as GUGCAUGCGCCUUAUGAAGCU 5′

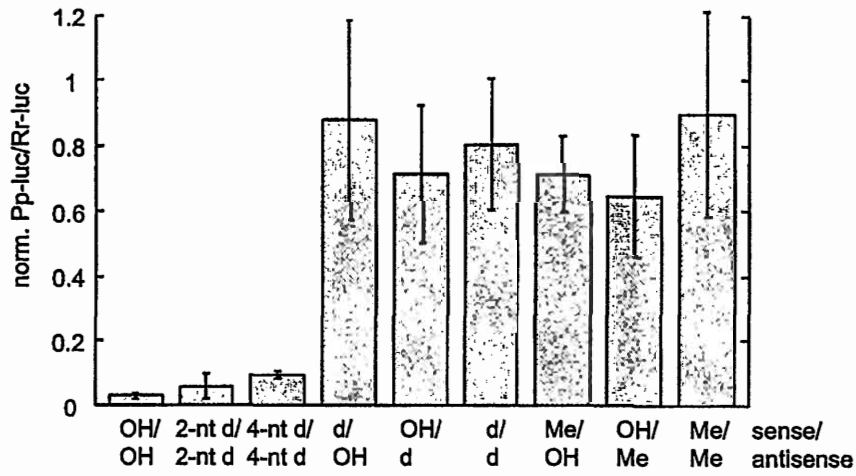
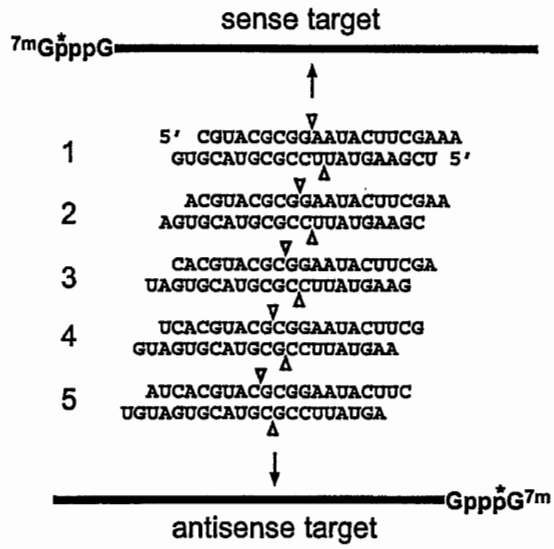


Fig. 15

A


sense target
7mG*pppG
1
5' CGUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
2
ACGUACGCGGAAUACUUCGAA
AGUGCAUGCGCCUUAUGAAGC
3
CACGUACGCGGAAUACUUCGA
UAGUGCAUGCGCCUUAUGAAG
4
UCACGUACGCGGAAUACUUCG
GUAGUGCAUGCGCCUUAUGAA
5
AUCACGUACGCGGAAUACUUC
UGUAGUGCAUGCGCCUUAUGA
GppG*G7m
antisense target

B

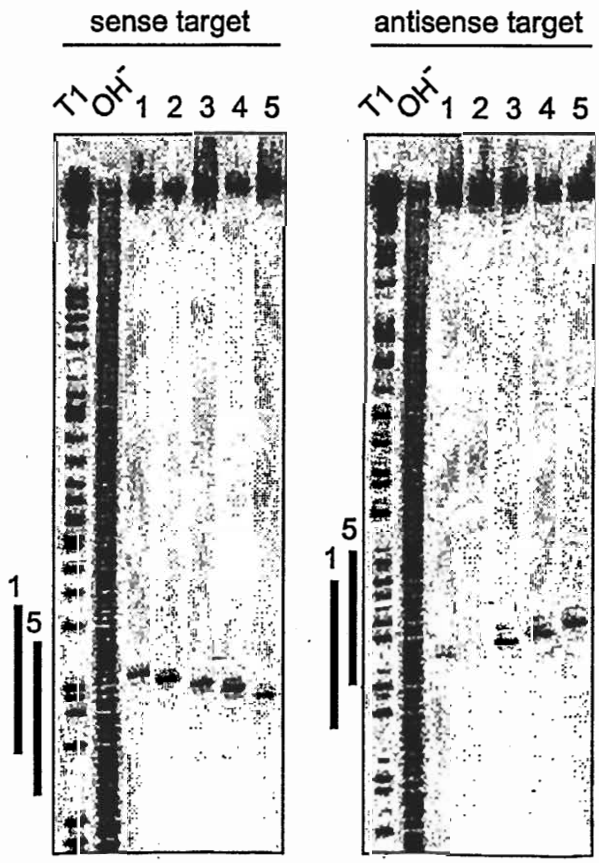

sense target
antisense target
T1
OH-
1 2 3 4 5
1
5

Fig. 16

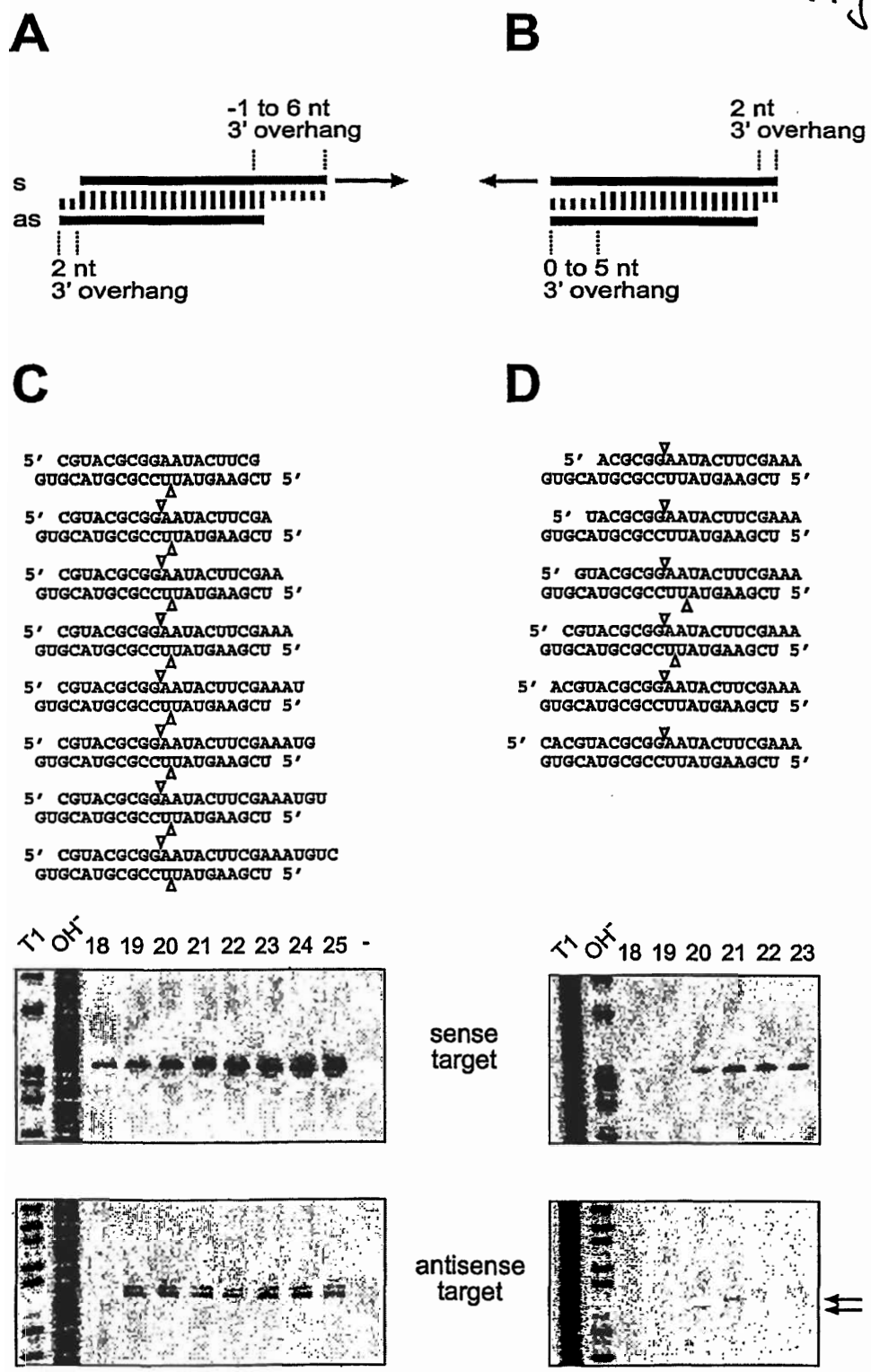
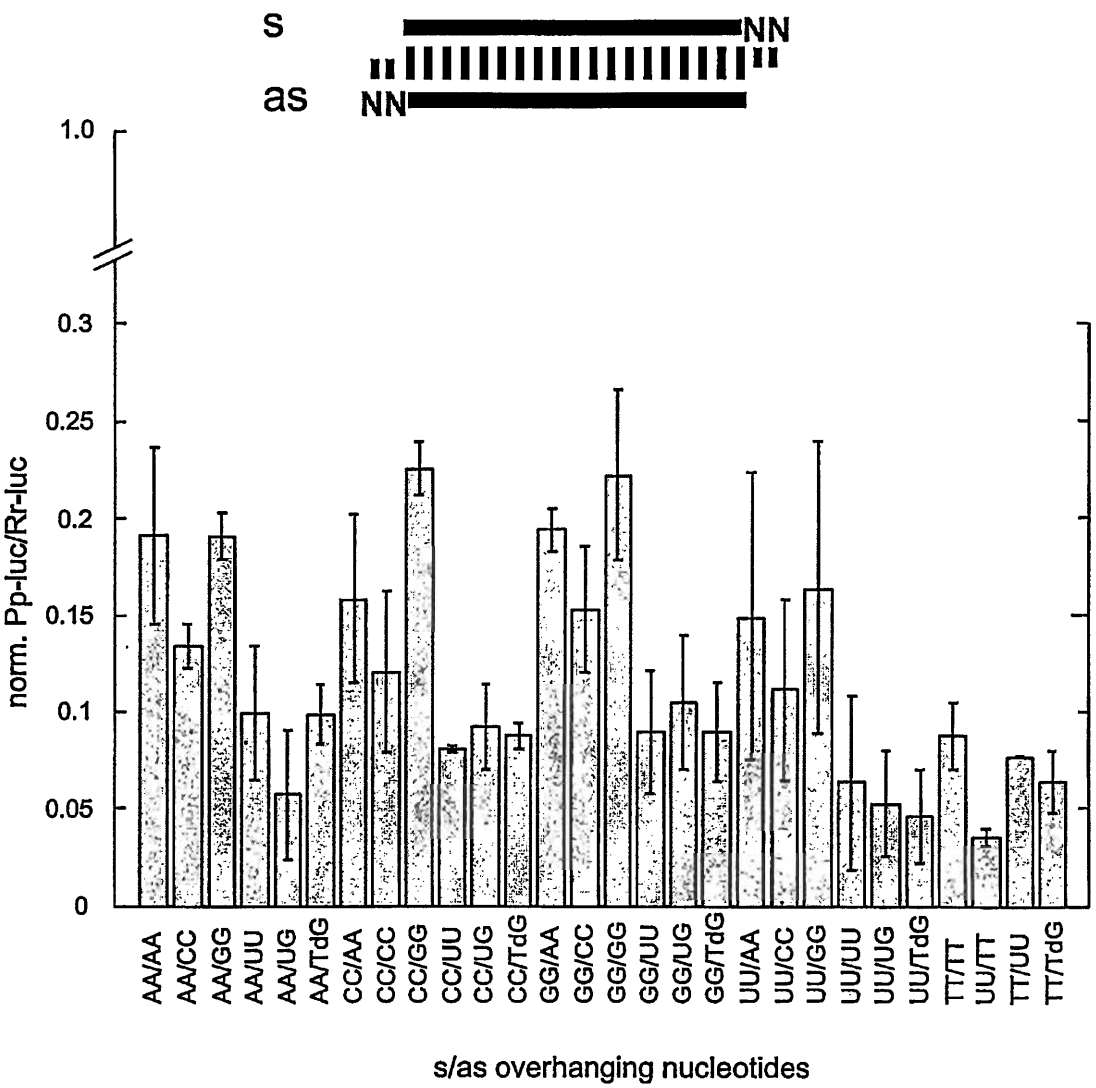

A
-1 to 6 nt
3' overhang
s
as
2 nt
3' overhang
B
2 nt
3' overhang
0 to 5 nt
3' overhang
C
5' CGUACGCGGAAUACUUCG
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGA
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAAU
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAAUG
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAAUGU
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAAUGUC
GUGCAUGCGCCUUAUGAAGCU 5'
D
5' ACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' UACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' GUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' CGUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' ACGUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
5' CACGUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5'
T1 OH- 18 19 20 21 22 23 24 25 -
T1 OH- 18 19 20 21 22 23
sense target
antisense target

Fig. 17


s
NN
as
NN
1.0
0.3
0.25
0.2
0.15
0.1
0.05
0
norm. Pp-luc/Rr-luc
AA/AA
AA/CC
AA/GG
AA/UU
AA/UG
AA/TdG
CC/AA
CC/CC
CC/GG
CC/UU
CC/UG
CC/TdG
GG/AA
GG/CC
GG/GG
GG/UU
GG/UG
GG/TdG
UU/AA
UU/CC
UU/GG
UU/UU
UU/UG
UU/TdG
TT/TT
UU/TT
TT/UU
TT/TdG
s/as overhanging nucleotides

31 MAY 2005

Fig. 18

ref 5′ CGUACGCGGAAUACUUCGATT
TTGCAUGCGCCUUAUGAAGCU 5′

1 5′ AUGCCGCGGAAUACUUCGATT
TTUACGGCGCCUUAUGAAGCU 5′

2 5′ CGUAGCGCGAAUACUUCGATT
TTGCAUCGCGCUUAUGAAGCU 5′

3 5′ CGUACGCGAGUAACUUCGATT
TTGCAUGCGCUCAUUGAAGCU 5′

4 5′ CGUACGCGGAAUUUCACGATT
TTGCAUGCGCCUUAAAGUGCU 5′

5 5′ CGUACGCGGAAUACUUAGCTT
TTGCAUGCGCCUUAUGAAUCG 5′

6 5′ CGUACGCGGUAUACUUCGATT
TTGCAUGCGCCAUAUGAAGCU 5′

7 5′ CGUACGCGGAUUACUUCGATT
TTGCAUGCGCCUAAUGAAGCU 5′

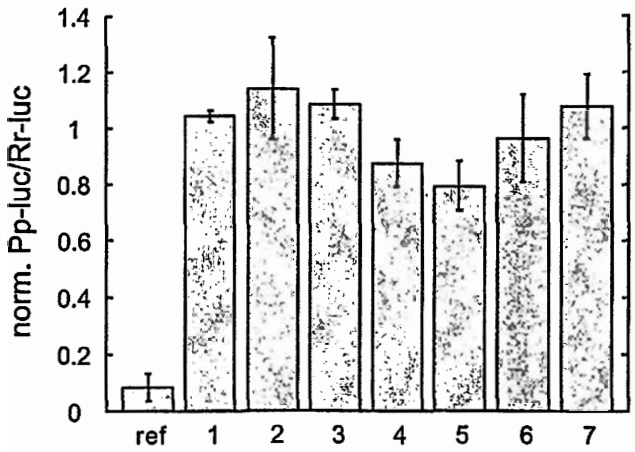
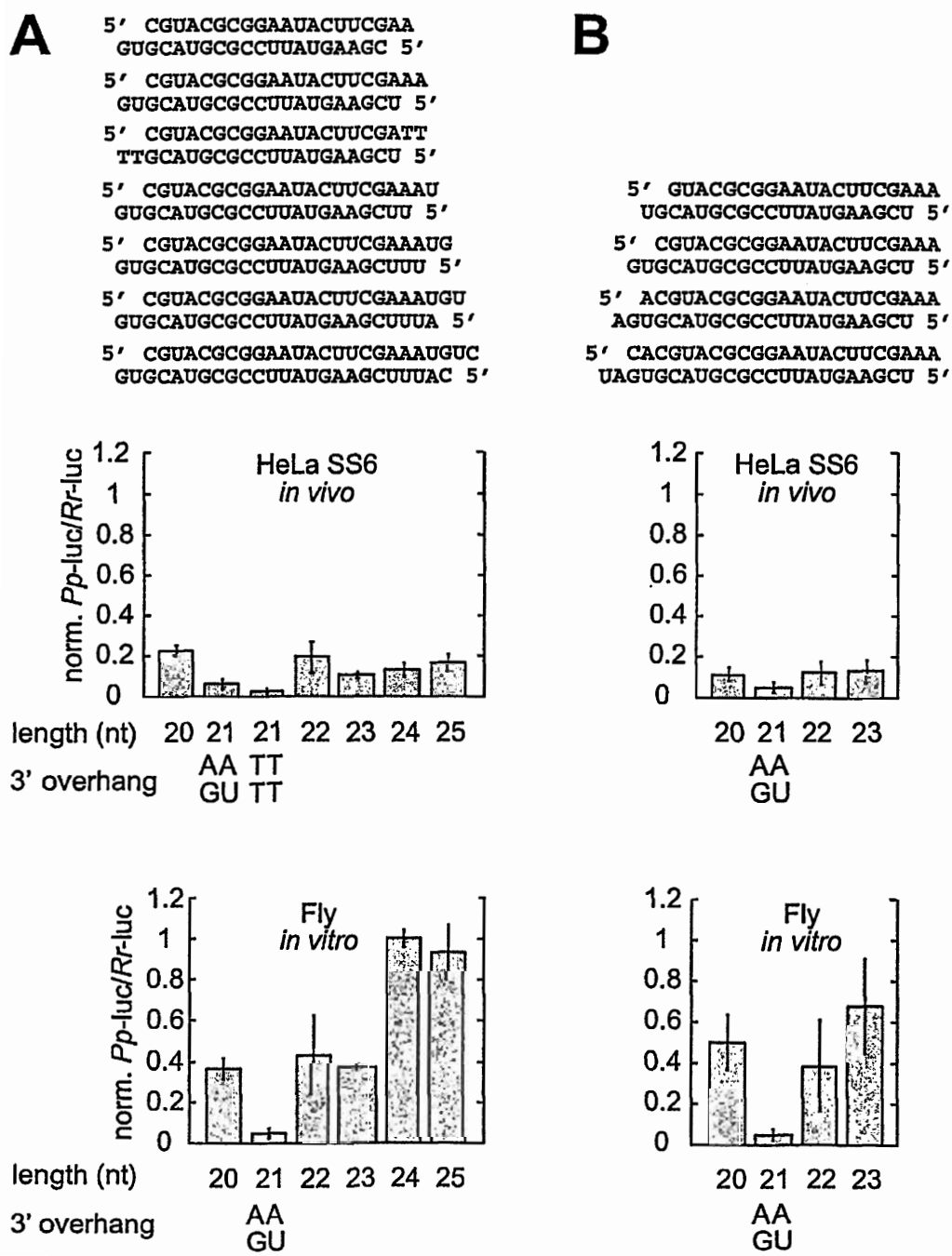


Figure 19


A
5′ CGUACGCGGAAUACUUCGAA
GUGCAUGCGCCUUAUGAAGC 5′
5′ CGUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5′
5′ CGUACGCGGAAUACUUCGATT
TTGCAUGCGCCUUAUGAAGCU 5′
5′ CGUACGCGGAAUACUUCGAAAU
GUGCAUGCGCCUUAUGAAGCUU 5′
5′ CGUACGCGGAAUACUUCGAAAUG
GUGCAUGCGCCUUAUGAAGCUUU 5′
5′ CGUACGCGGAAUACUUCGAAAUGU
GUGCAUGCGCCUUAUGAAGCUUUA 5′
5′ CGUACGCGGAAUACUUCGAAAUGUC
GUGCAUGCGCCUUAUGAAGCUUUAC 5′
B
5′ GUACGCGGAAUACUUCGAAA
UGCAUGCGCCUUAUGAAGCU 5′
5′ CGUACGCGGAAUACUUCGAAA
GUGCAUGCGCCUUAUGAAGCU 5′
5′ ACGUACGCGGAAUACUUCGAAA
AGUGCAUGCGCCUUAUGAAGCU 5′
5′ CACGUACGCGGAAUACUUCGAAA
UAGUGCAUGCGCCUUAUGAAGCU 5′
norm. Pp-luc/Rr-luc
HeLa SS6
in vivo
1.2
1
0.8
0.6
0.4
0.2
0
length (nt) 20 21 21 22 23 24 25
3′ overhang AA TT
GU TT
HeLa SS6
in vivo
20 21 22 23
AA
GU
norm. Pp-luc/Rr-luc
Fly
in vitro
length (nt) 20 21 22 23 24 25
3′ overhang AA
GU
Fly
in vitro
20 21 22 23
AA
GU