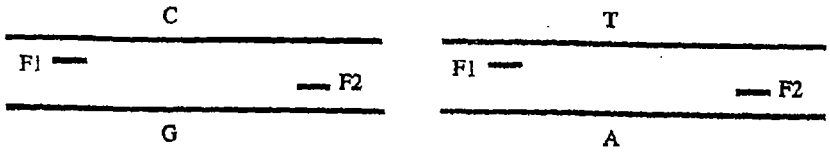
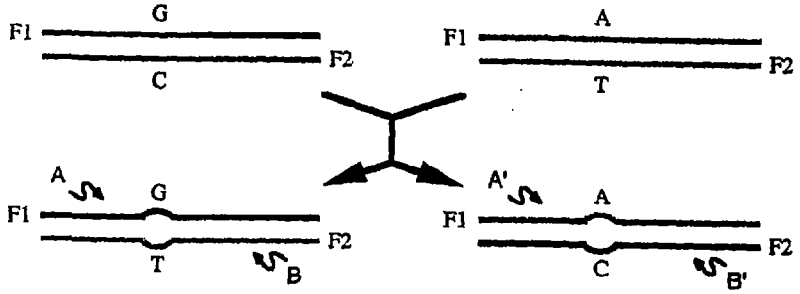
Mismatch scanning Assay. (Endo V / DNA Ligase)

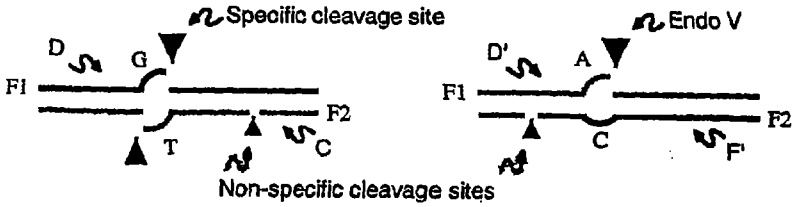
1. PCR amplify gene using primers with different fluorescent labels and *Taq* DNA polymerase.



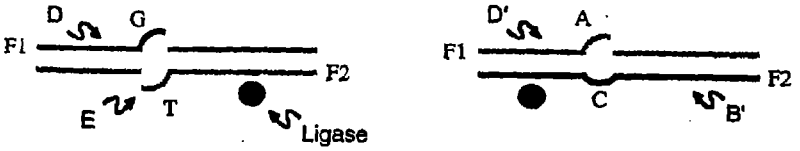
2. Denature and reanneal PCR products to form heteroduplexed DNA. (Homoduplexed products not shown).



3. Preferentially nick DNA one base to the 3' side of mismatches using thermostable Endonuclease V.



4. Add thermostable ligase to re-seal background nicks at perfect match regions.



163163

	1.	2.	3.
EndoV	-	+	+
Ligase	-	-	+

5. Separate fluorescent products on a DNA sequencer (using length standards) to approximate site of mismatch.

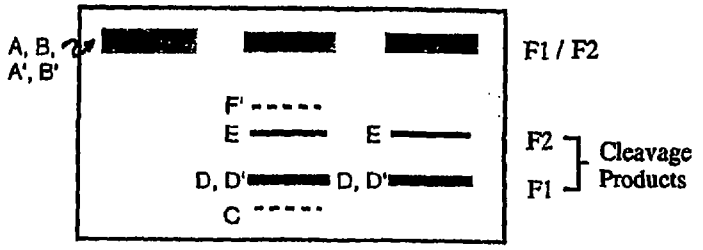
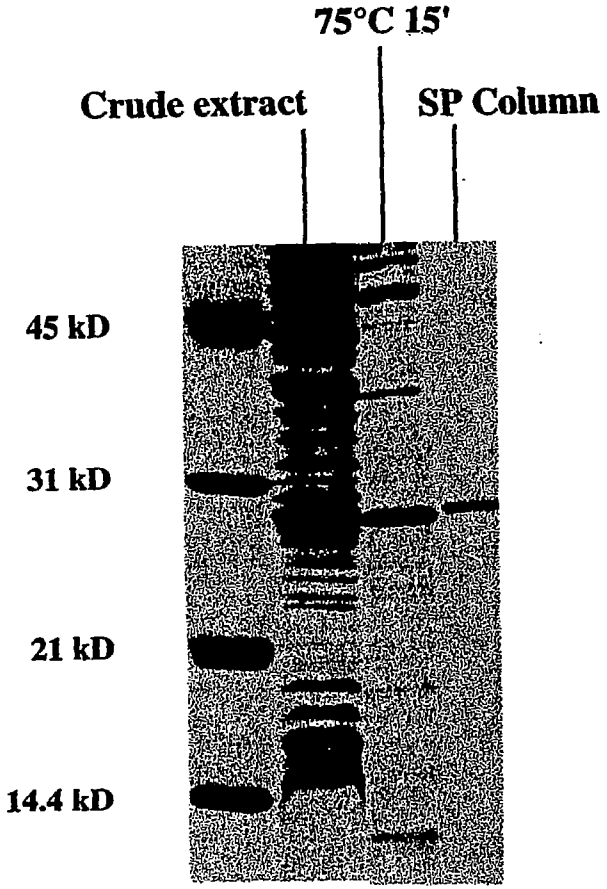
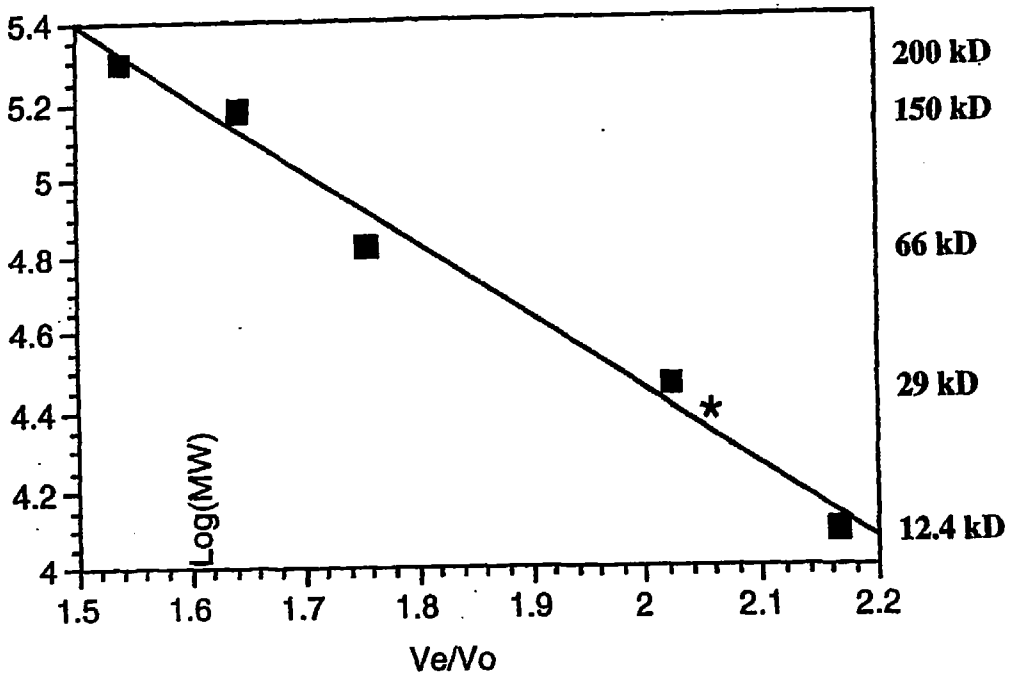


Figure 1

G00002


75°C 15'
Crude extract
SP Column
45 kD
31 kD
21 kD
14.4 kD

A


5.4
5.2
5
4.8
4.6
4.4
4.2
4
Log(MW)
1.5
1.6
1.7
1.8
1.9
2
2.1
2.2
Ve/Vo
200 kD
150 kD
66 kD
29 kD
12.4 kD

B

Figure 2

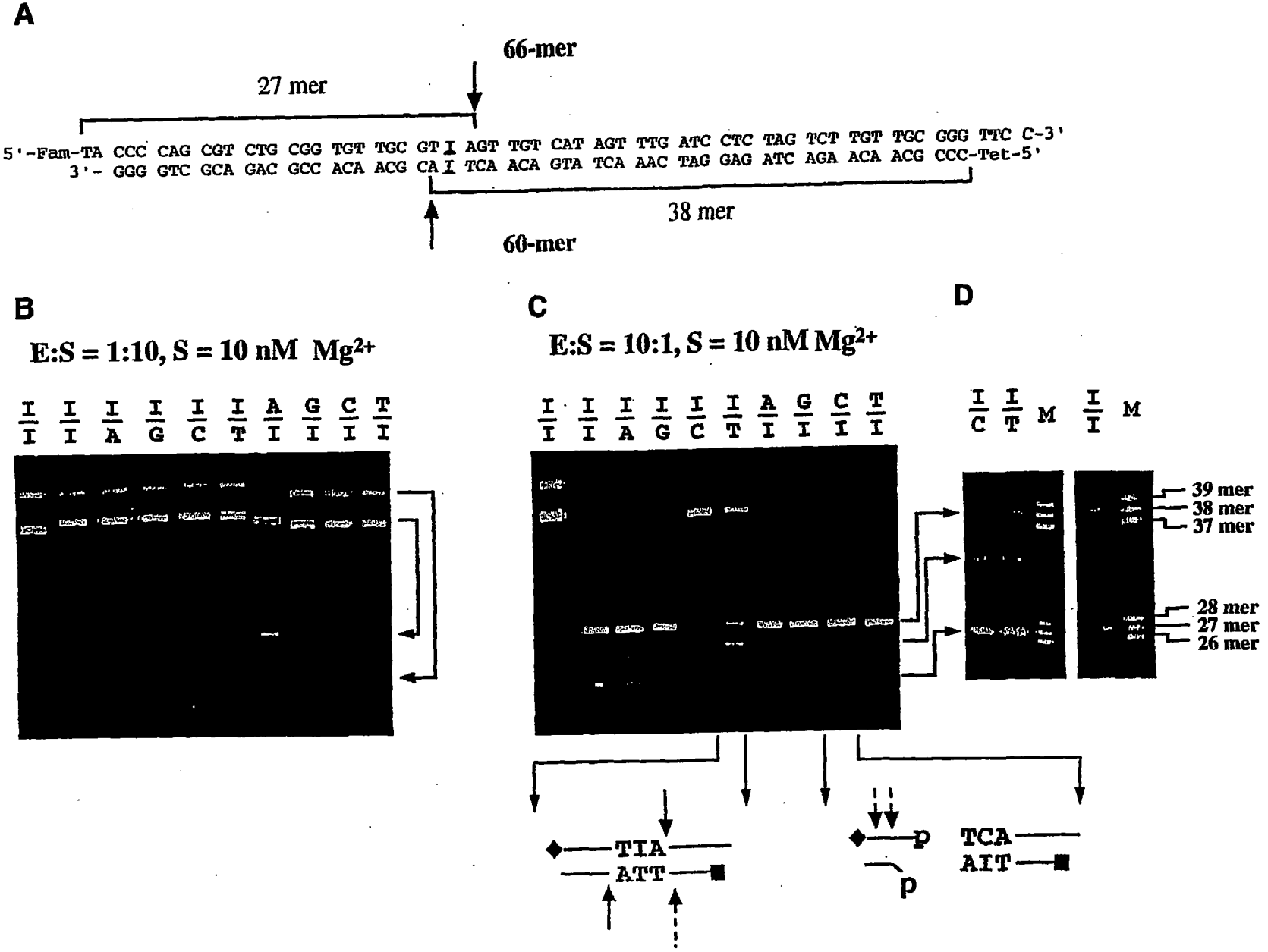
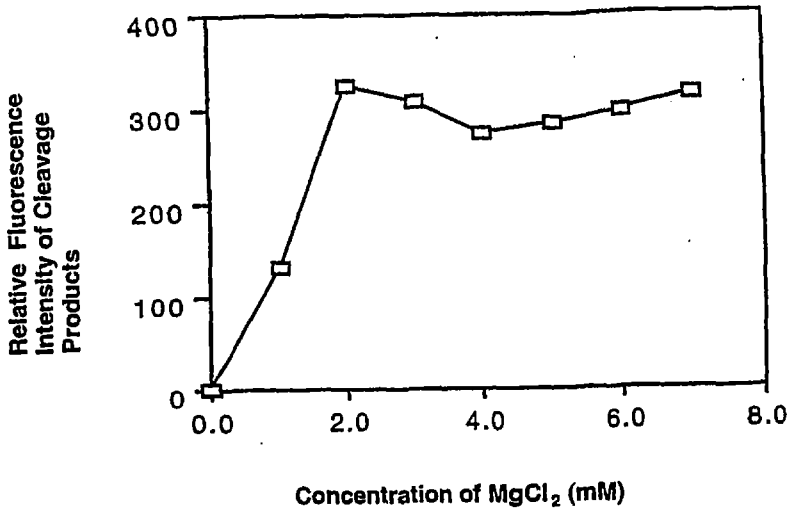

A
66-mer
27 mer
5'-Fam-TA CCC CAG CGT CTG CGG TGT TGC GT I AGT TGT CAT AGT TTG ATC CTC TAG TCT TGT TGC GGG TTC C-3'
3'- GGG GTC GCA GAC GCC ACA ACG CA I TCA ACA GTA TCA AAC TAG GAG ATC AGA ACA ACG CCC-Tet-5'
38 mer
60-mer
B
E:S = 1:10, S = 10 nM Mg2+
I/I I/I A/I G/I C/I T/I A/I G/I C/I T/I
C
E:S = 10:1, S = 10 nM Mg2+
I/I I/I A/I G/I C/I T/I A/I G/I C/I T/I
D
I/C I/T M I/I M
39 mer
38 mer
37 mer
28 mer
27 mer
26 mer
TIA
ATT
p
p
TCA
AIT

Figure 3

A


Relative Fluorescence Intensity of Cleavage Products
400
300
200
100
0
0.0
2.0
4.0
6.0
8.0
Concentration of MgCl2 (mM)

B

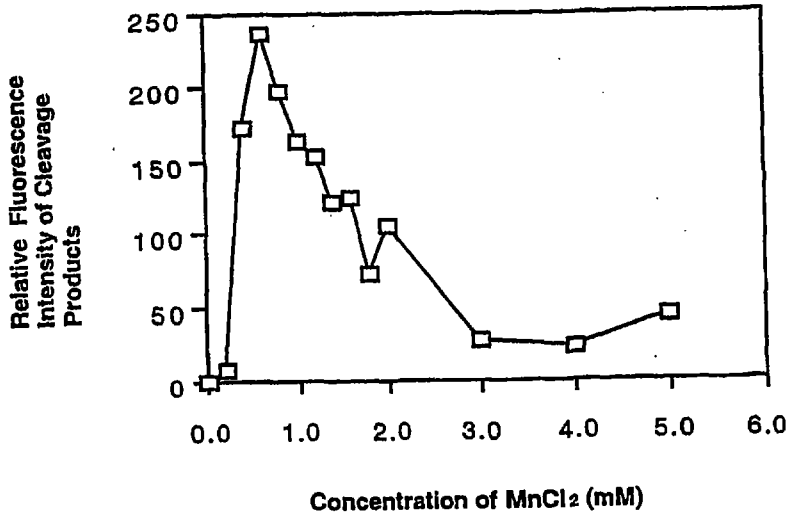

Relative Fluorescence Intensity of Cleavage Products
250
200
150
100
50
0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
Concentration of MnCl2 (mM)

Figure 4

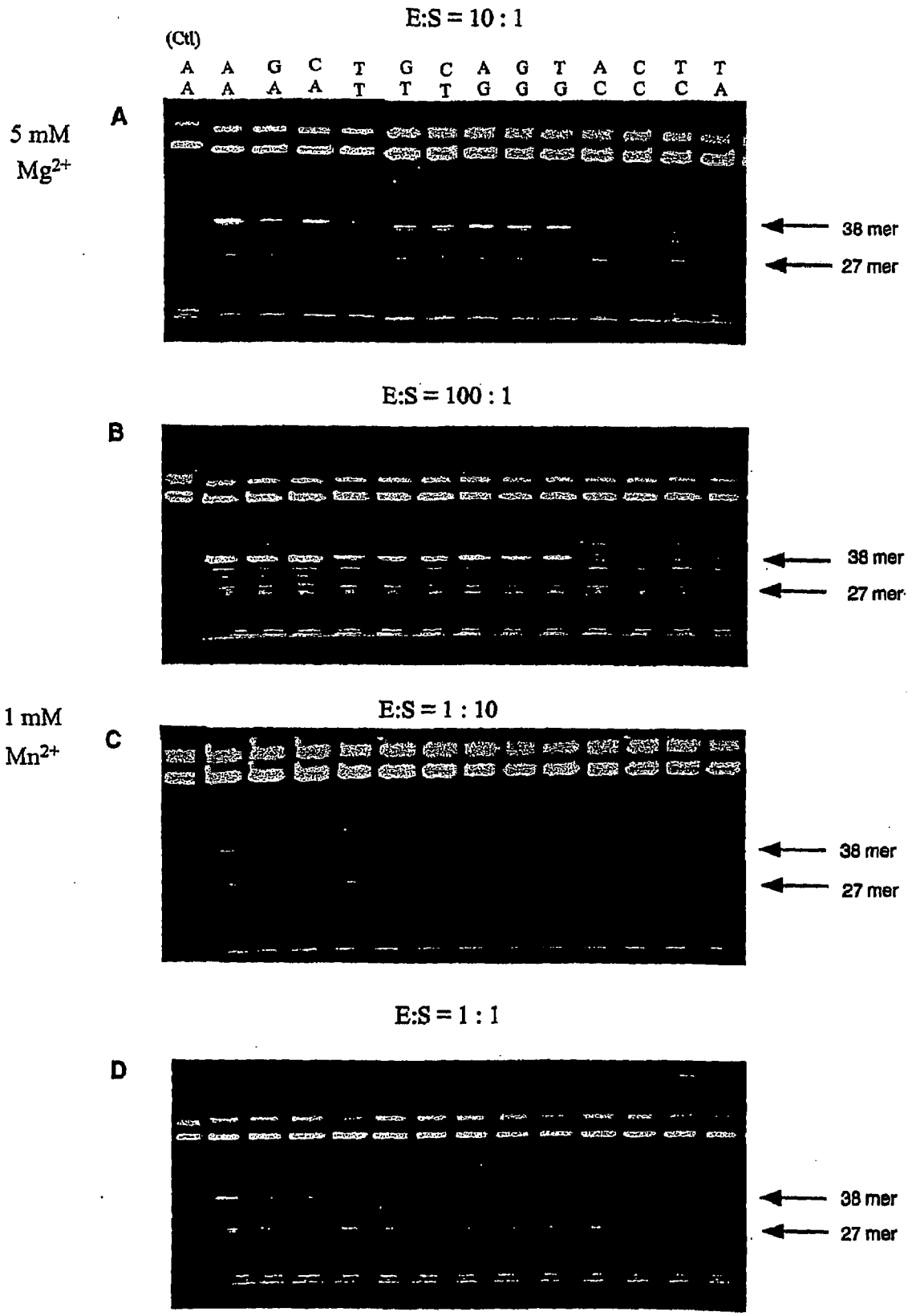

E:S = 10 : 1
(Ctl)
A A G C T G C A G T A C T T
A A A A T T G G G C C C A
5 mM
Mg2+
A
38 mer
27 mer
E:S = 100 : 1
B
38 mer
27 mer
1 mM
Mn2+
E:S = 1 : 10
C
38 mer
27 mer
E:S = 1 : 1
D
38 mer
27 mer

Figure 5

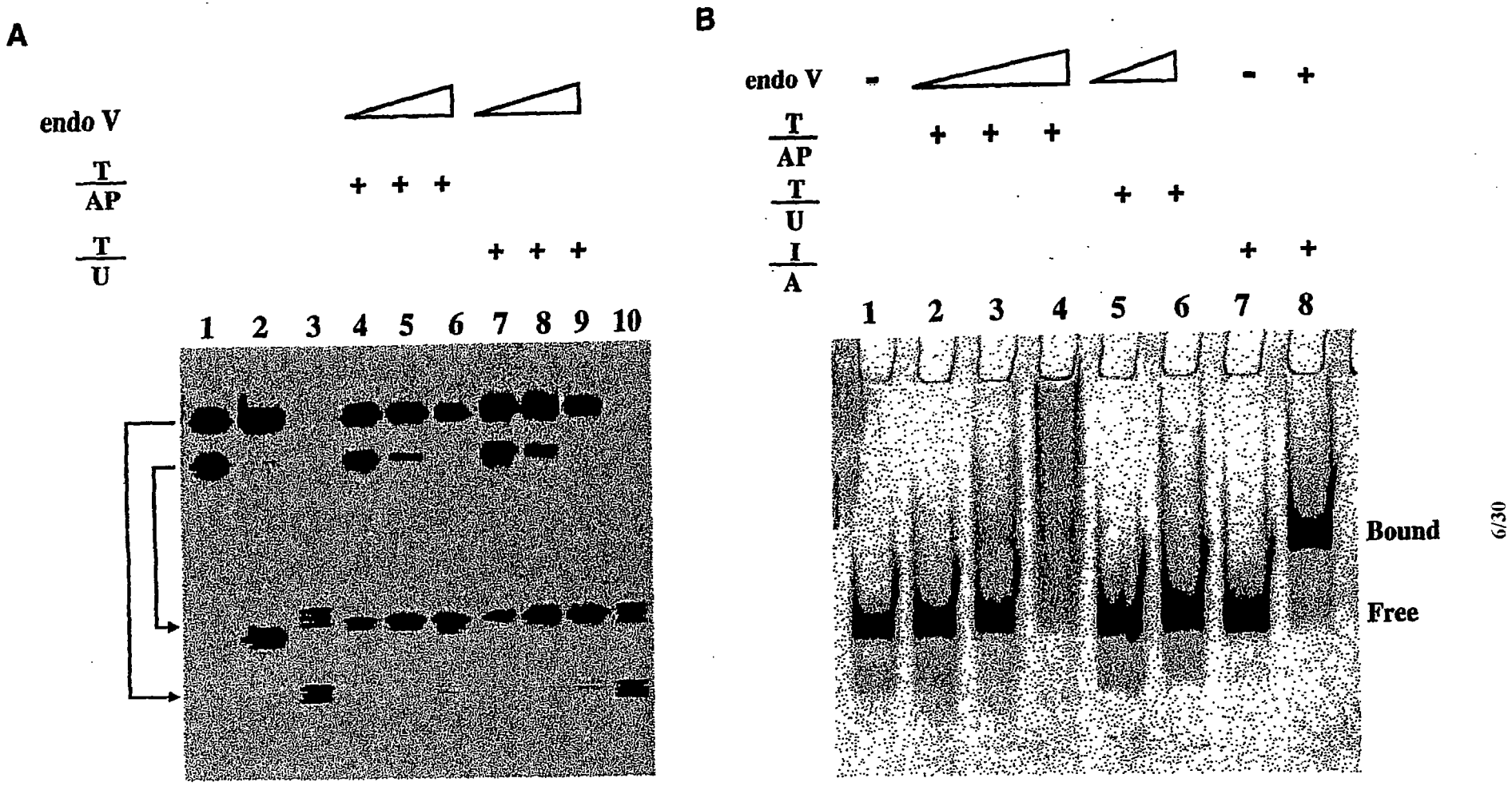

A
endo V
T
AP
T
U
1 2 3 4 5 6 7 8 9 10
B
endo V
T
AP
T
U
I
A
1 2 3 4 5 6 7 8
Bound
Free

Figure 6

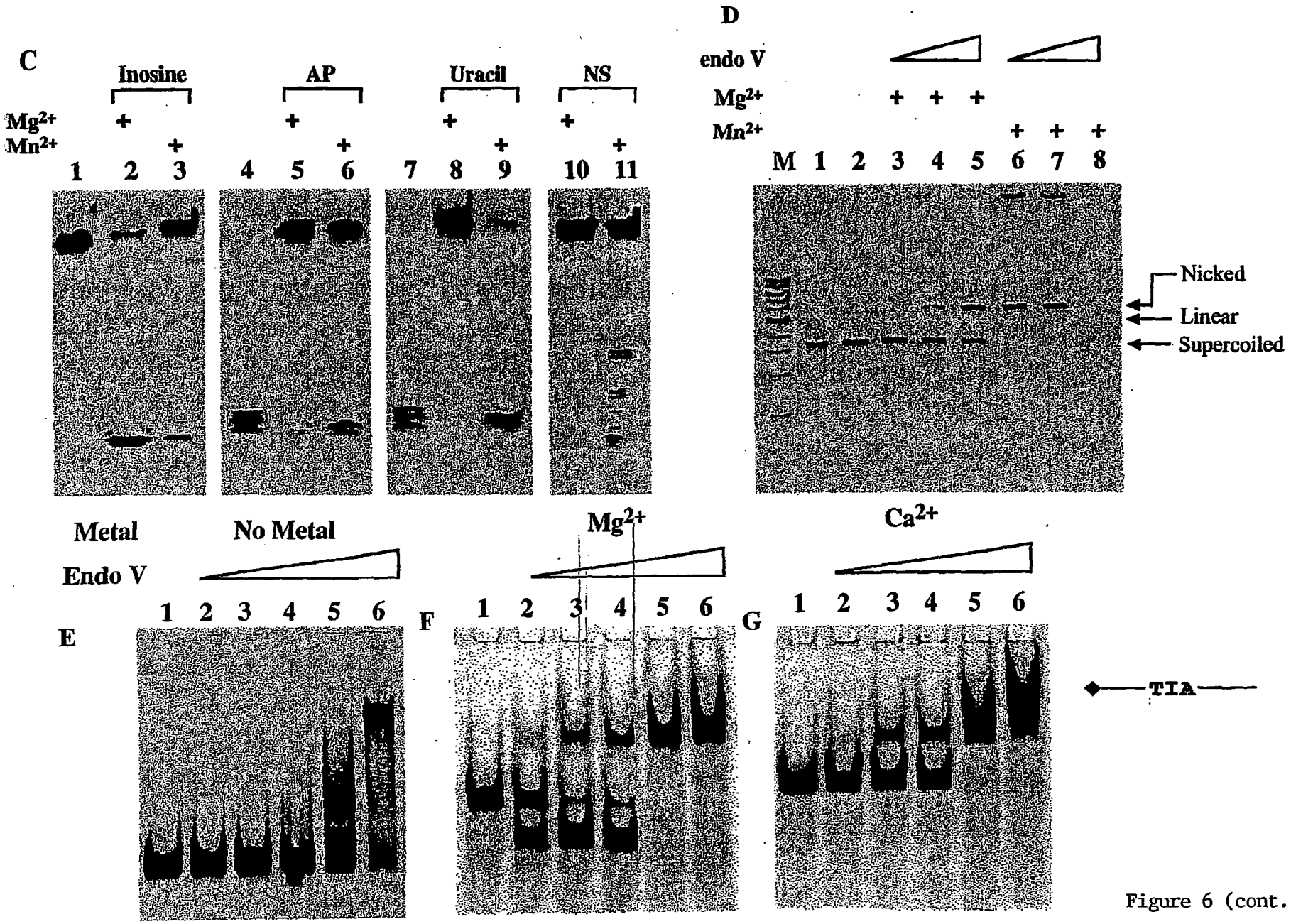


Figure 6 (cont.)

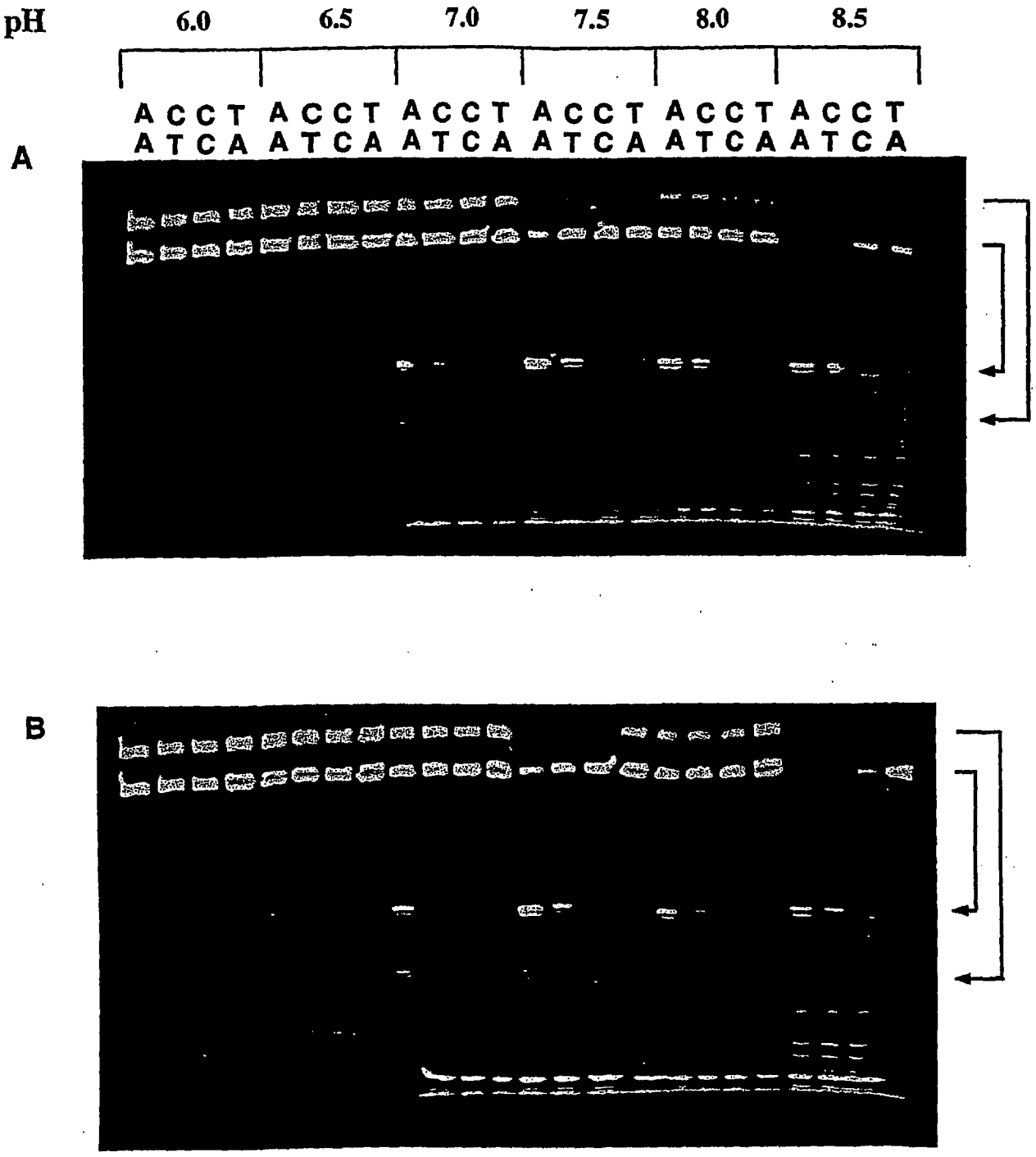

pH
6.0
6.5
7.0
7.5
8.0
8.5
A
A C C T A C C T A C C T A C C T A C C T A C C T
A T C A A T C A A T C A A T C A A T C A A T C A
B

Figure 7.

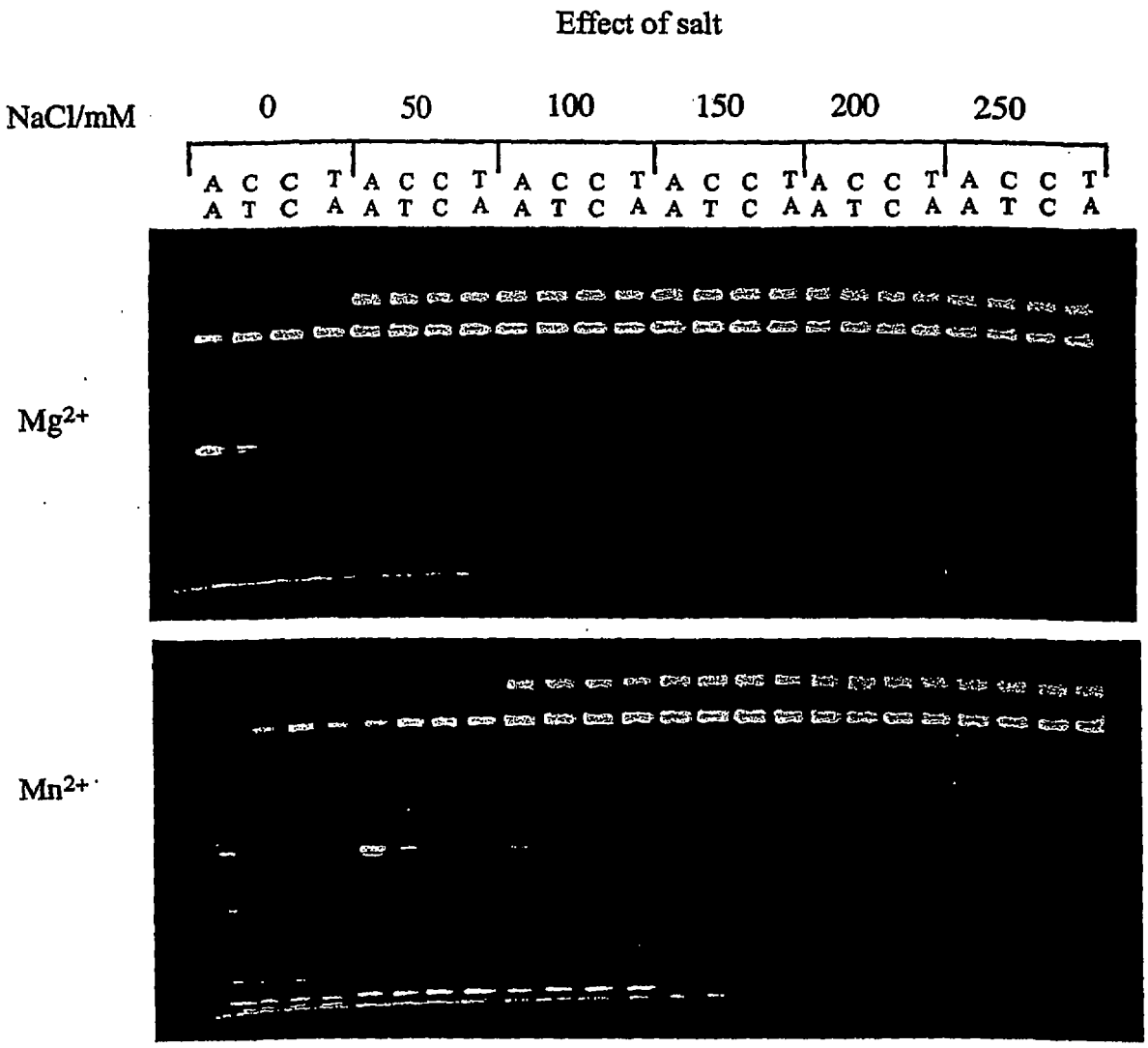

Effect of salt
NaCl/mM
0
50
100
150
200
250
A C C T A C C T A C C T A C C T A C C T A C C T
A T C A A T C A A T C A A T C A A T C A A T C A
Mg2+
Mn2+

Figure 8

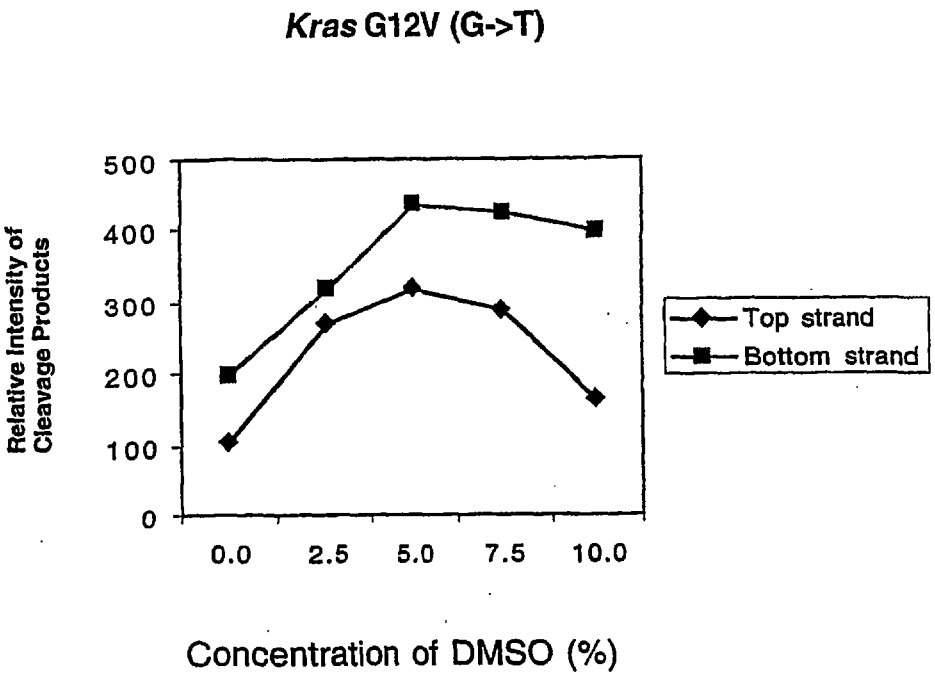
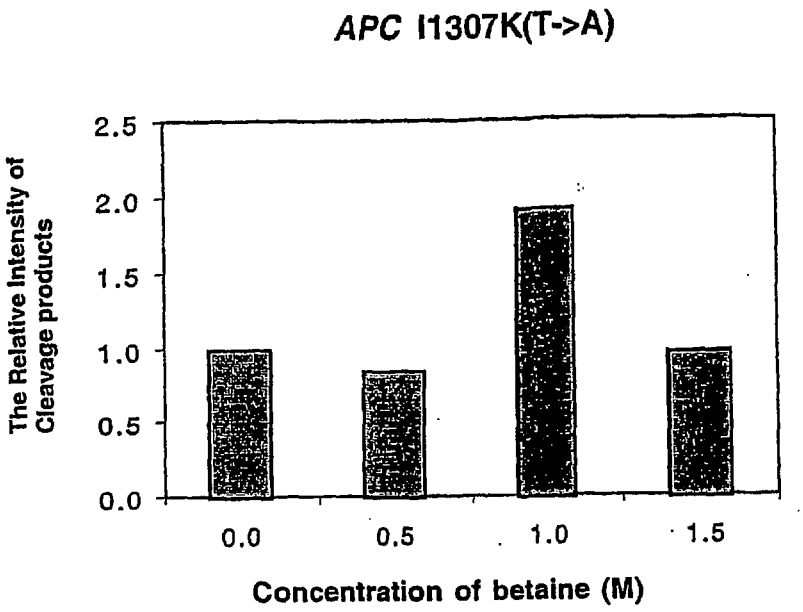

Kras G12V (G->T)
Relative Intensity of Cleavage Products
500
400
300
200
100
0
0.0
2.5
5.0
7.5
10.0
Concentration of DMSO (%)
Top strand
Bottom strand

Figure 9

A


APC I1307K(T->A)
The Relative Intensity of Cleavage products
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.5
1.0
1.5
Concentration of betaine (M)

B

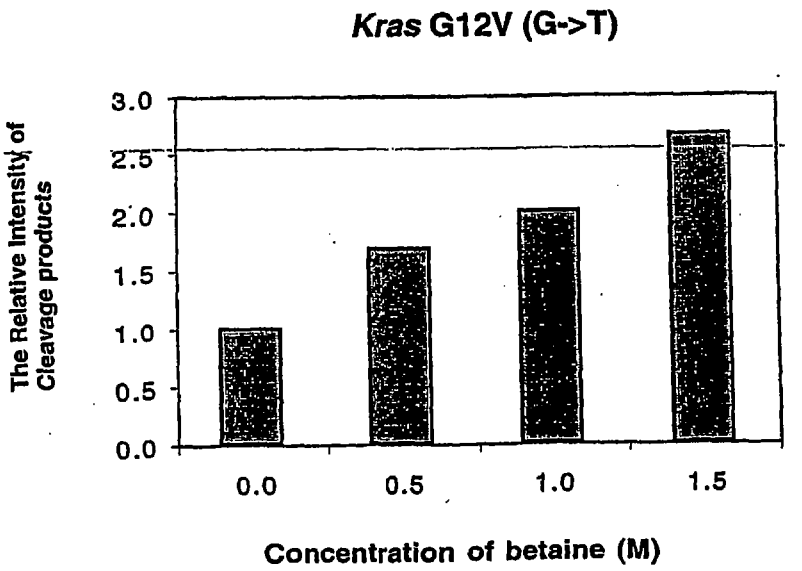
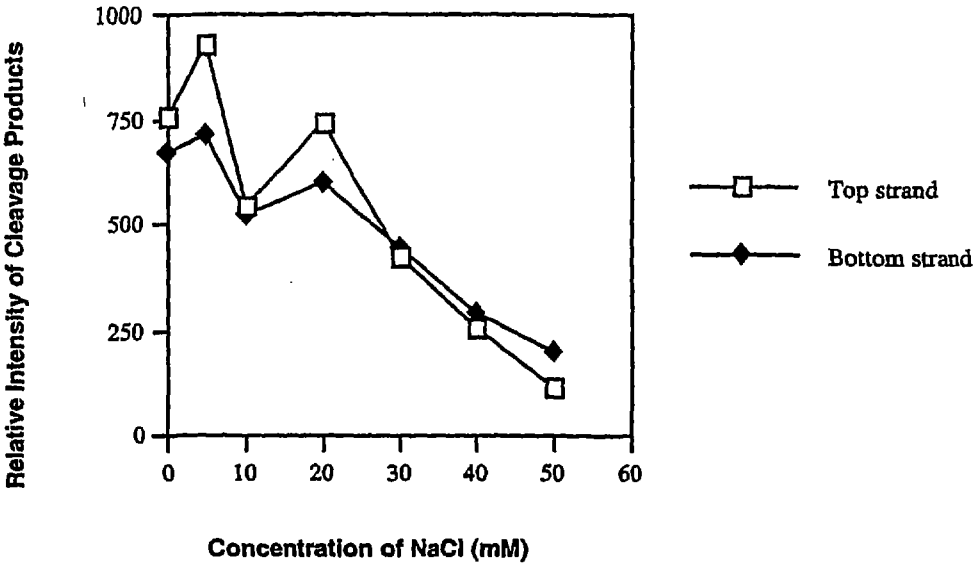

Kras G12V (G->T)
The Relative Intensity of Cleavage products
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.5
1.0
1.5
Concentration of betaine (M)

Figure 10

k-ras G12V (G->T)

A



B

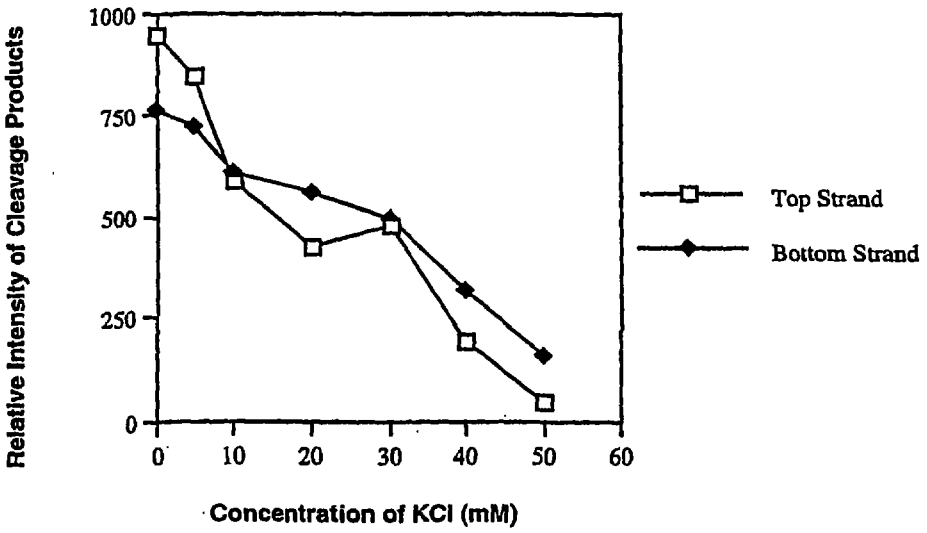


Figure 11

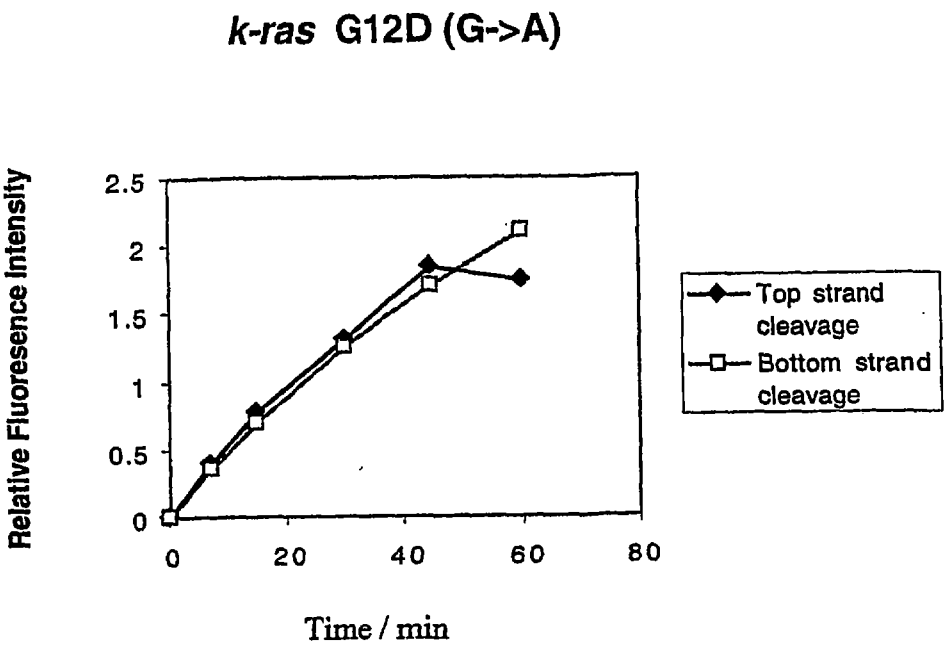

k-ras G12D (G->A)
Relative Fluoresence Intensity
2.5
2
1.5
1
0.5
0
0
20
40
60
80
Time / min
Top strand cleavage
Bottom strand cleavage

Figure 12

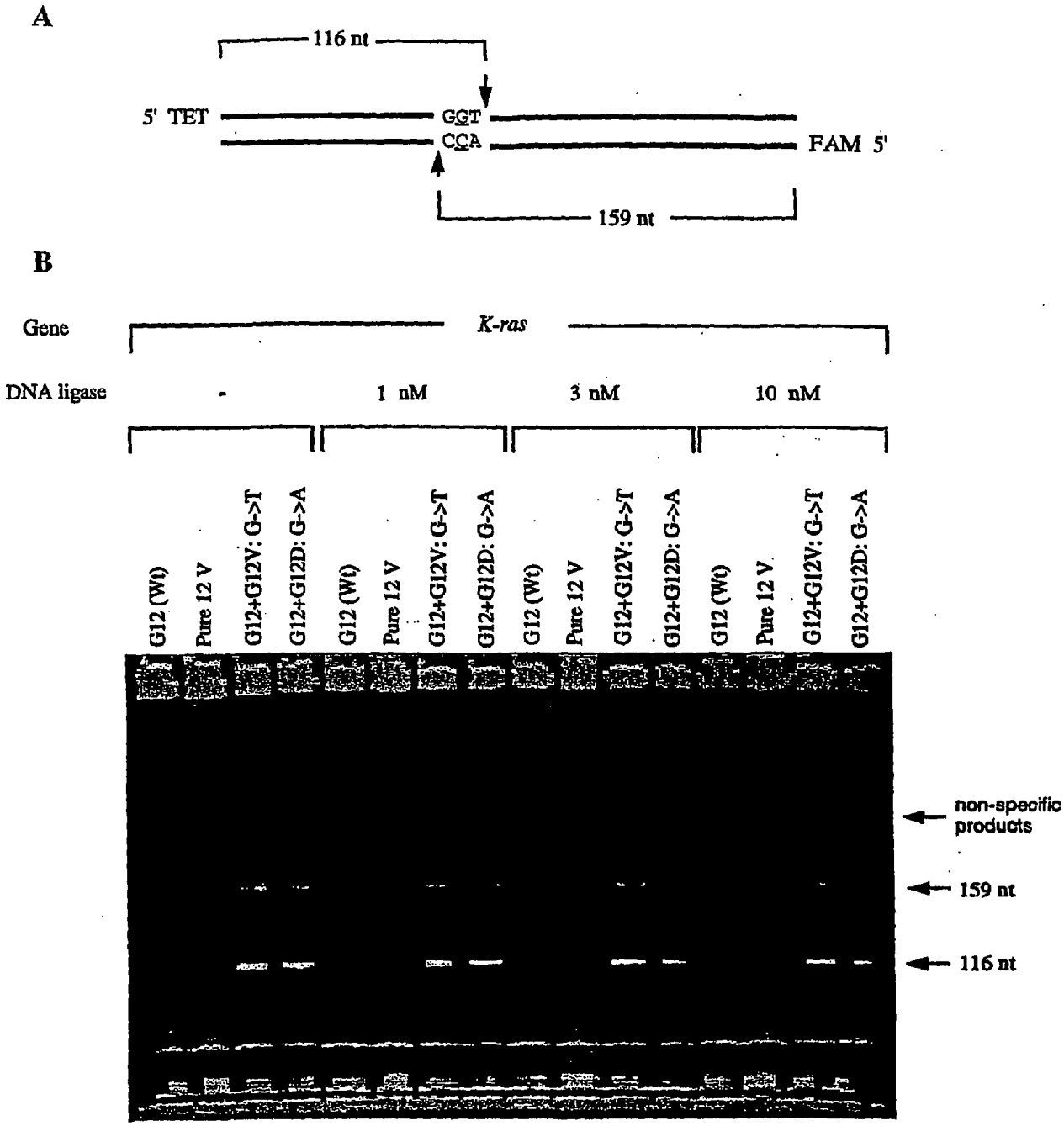

A
116 nt
5' TET
GGT
CCA
FAM 5'
159 nt
B
Gene
K-ras
DNA ligase
-
1 nM
3 nM
10 nM
G12 (Wt)
Pure 12 V
G12+G12V: G->T
G12+G12D: G->A
non-specific products
159 nt
116 nt

Figure 13

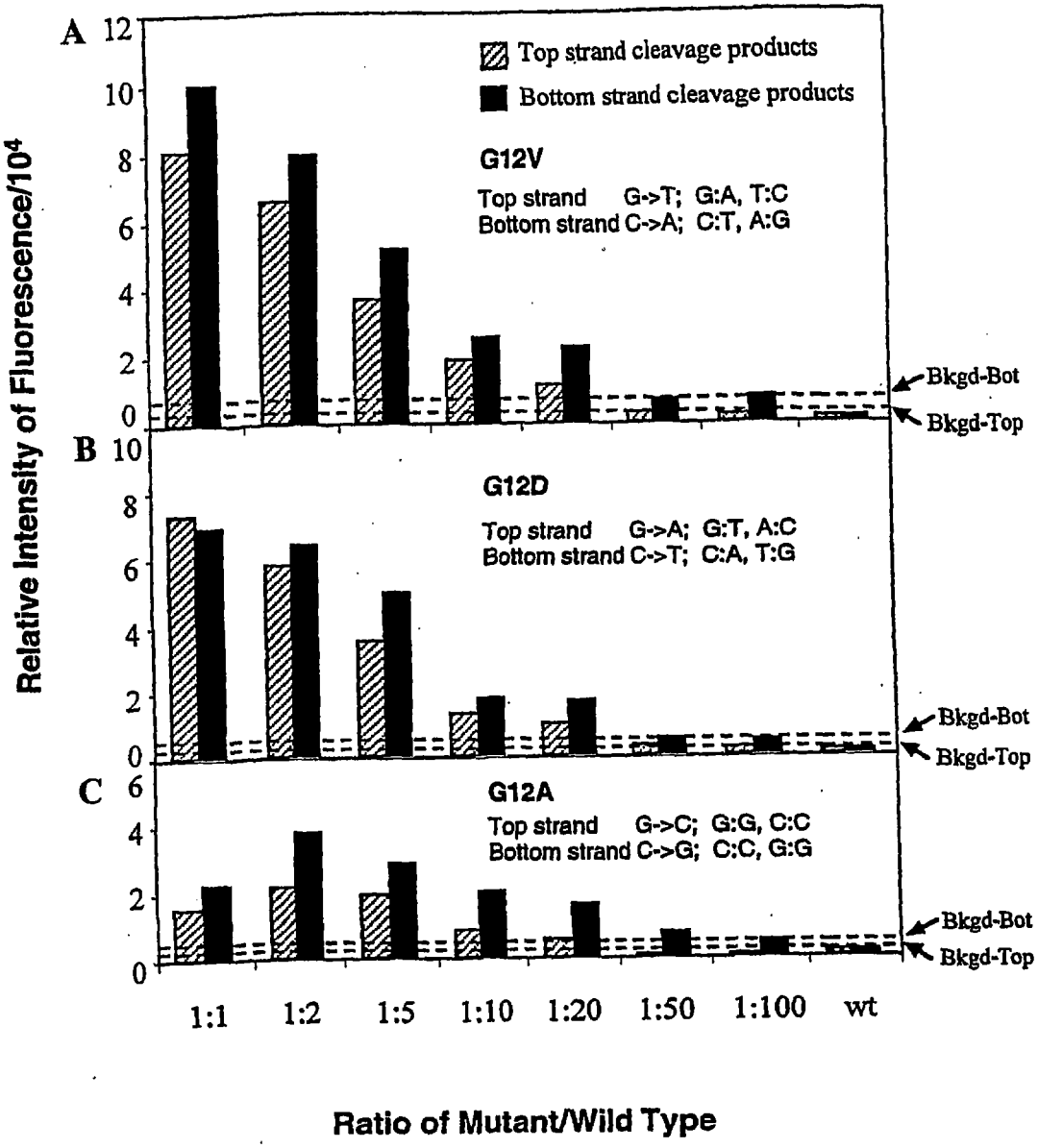

A
B
C
Top strand cleavage products
Bottom strand cleavage products
G12V
Top strand G->T; G:A, T:C
Bottom strand C->A; C:T, A:G
G12D
Top strand G->A; G:T, A:C
Bottom strand C->T; C:A, T:G
G12A
Top strand G->C; G:G, C:C
Bottom strand C->G; C:C, G:G
Bkgd-Bot
Bkgd-Top
Relative Intensity of Fluorescence/10^4
1:1
1:2
1:5
1:10
1:20
1:50
1:100
wt
Ratio of Mutant/Wild Type

Figure 14

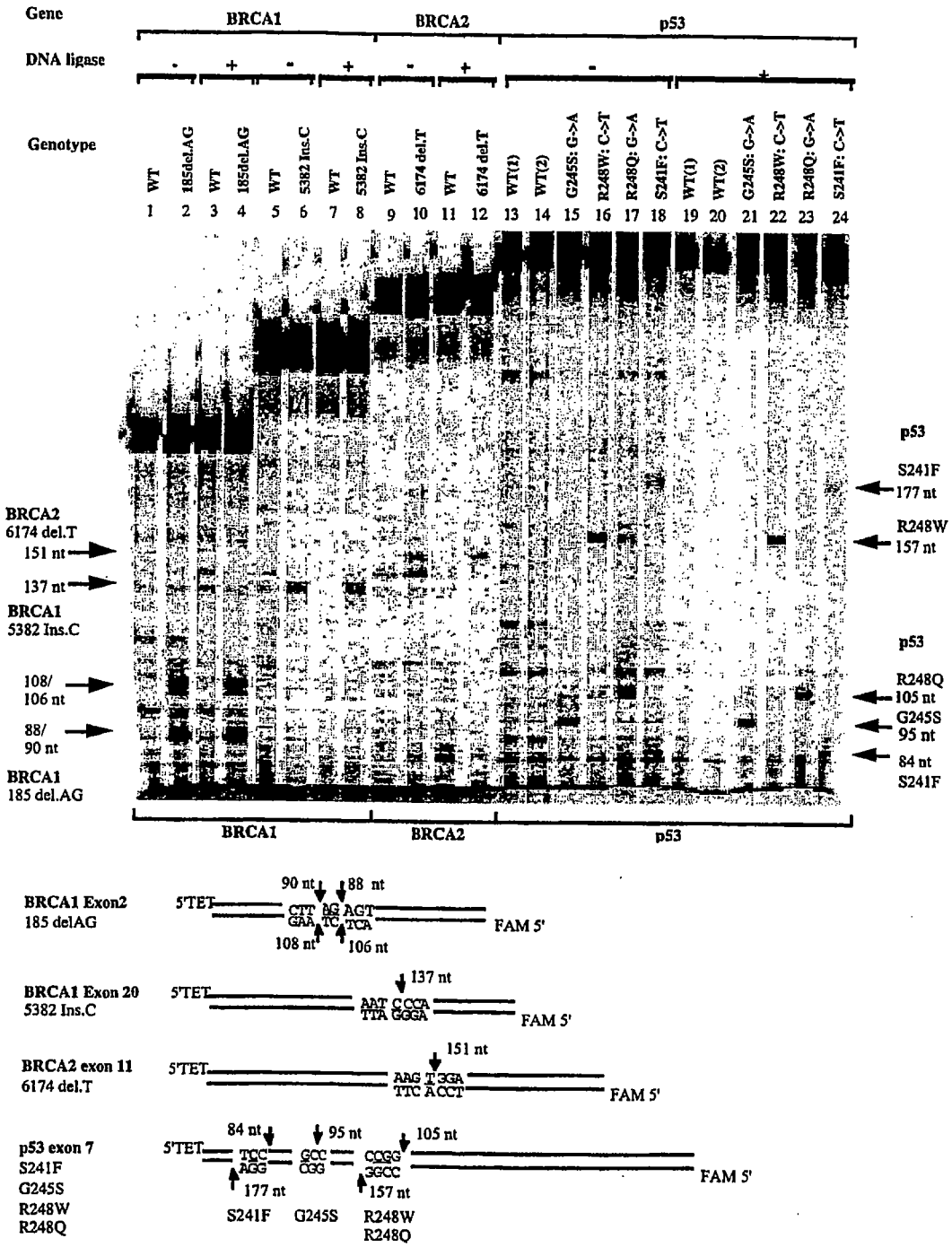

Gene
BRCA1
BRCA2
p53
DNA ligase
Genotype
WT
185del.AG
WT
185delAG
WT
5382 Ins.C
WT
5382 Ins.C
WT
6174 del.T
WT
6174 del.T
WT(1)
WT(2)
G245S: G->A
R248W: C->T
R248Q: G->A
S241F: C->T
WT(1)
WT(2)
G245S: G->A
R248W: C->T
R248Q: G->A
S241F: C->T
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
BRCA2
6174 del.T
151 nt
137 nt
BRCA1
5382 Ins.C
108/
106 nt
88/
90 nt
BRCA1
185 del.AG
p53
S241F
177 nt
R248W
157 nt
p53
R248Q
105 nt
G245S
95 nt
84 nt
S241F
BRCA1
BRCA2
p53
BRCA1 Exon2
185 delAG
5'TET
90 nt
88 nt
CTT AG AGT
GAA TC TCA
FAM 5'
108 nt
106 nt
BRCA1 Exon 20
5382 Ins.C
5'TET
137 nt
AAT CCCA
TTA GGGA
FAM 5'
BRCA2 exon 11
6174 del.T
5'TET
151 nt
AAG T GGA
TTC A CCT
FAM 5'
p53 exon 7
S241F
G245S
R248W
R248Q
5'TET
84 nt
95 nt
105 nt
TCC GCC CCGG
AGG CGG GGCC
FAM 5'
177 nt
157 nt
S241F
G245S
R248W
R248Q

Figure 15

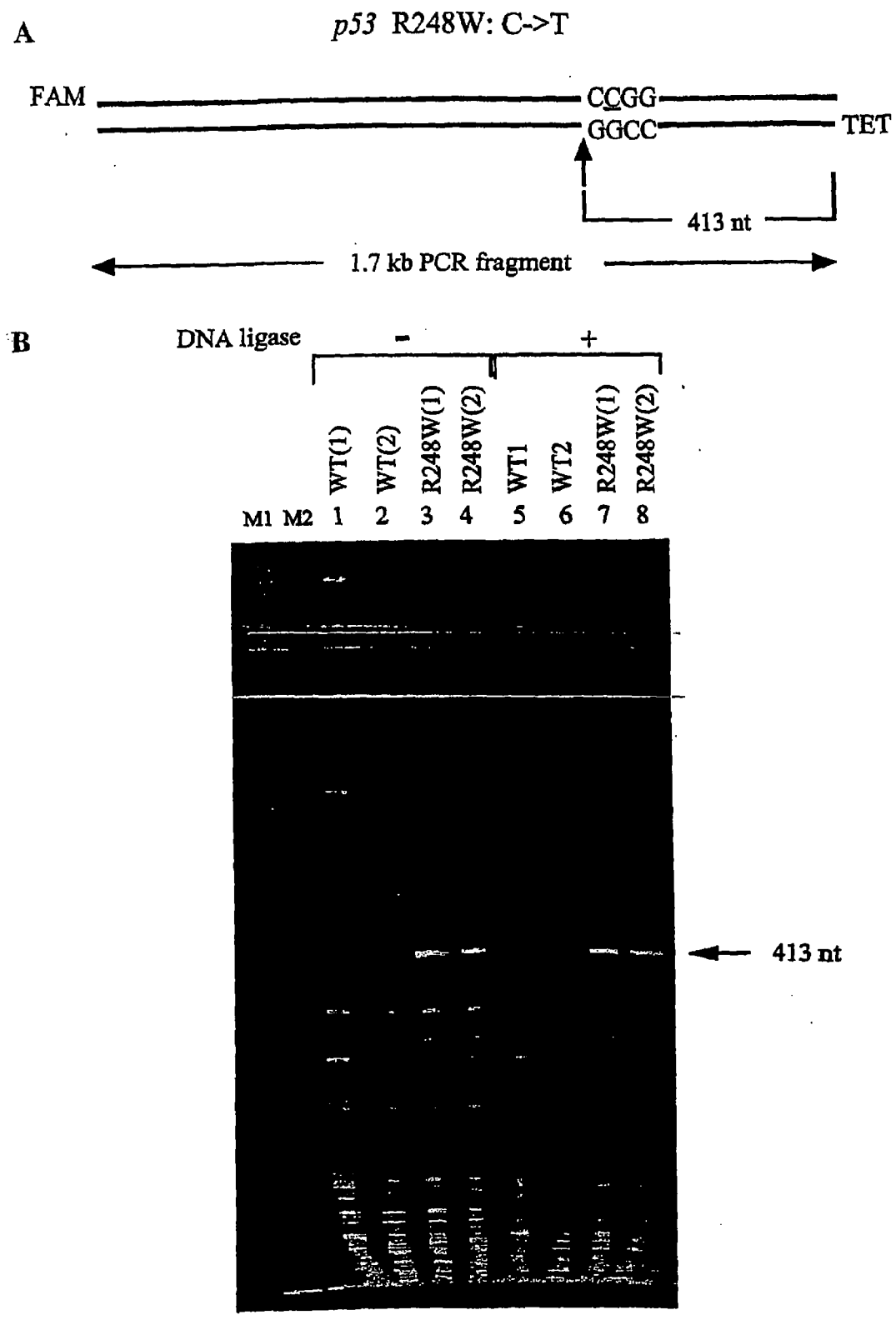
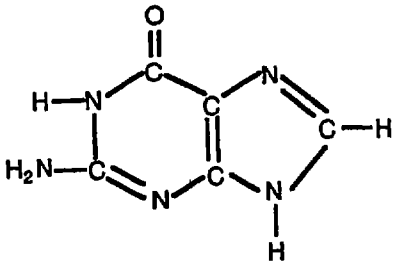

A
p53 R248W: C->T
FAM
CCGG
GGCC
TET
413 nt
1.7 kb PCR fragment
B
DNA ligase
-
+
WT(1)
WT(2)
R248W(1)
R248W(2)
WT1
WT2
R248W(1)
R248W(2)
M1 M2 1 2 3 4 5 6 7 8
413 nt

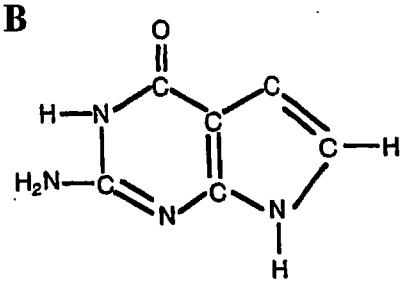
Figure 16

A



Guanine

B



7-deaza-Guanine

C

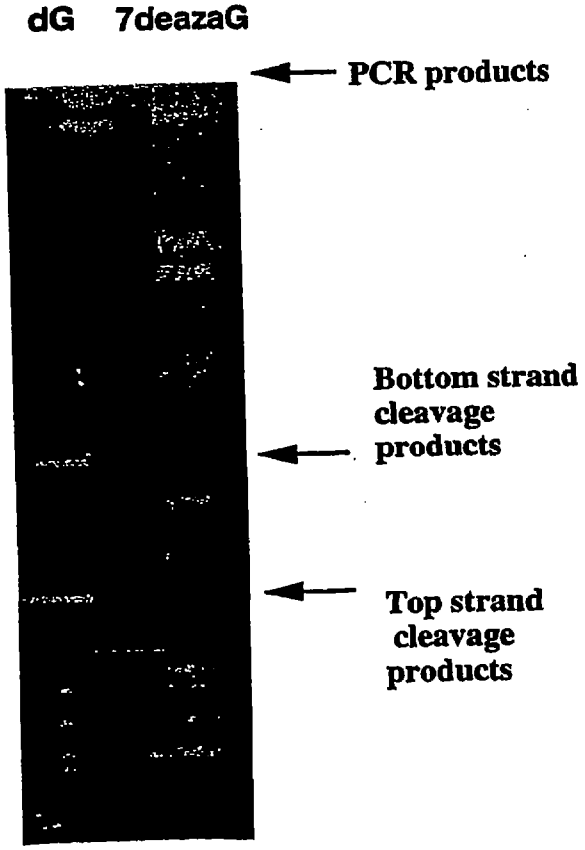
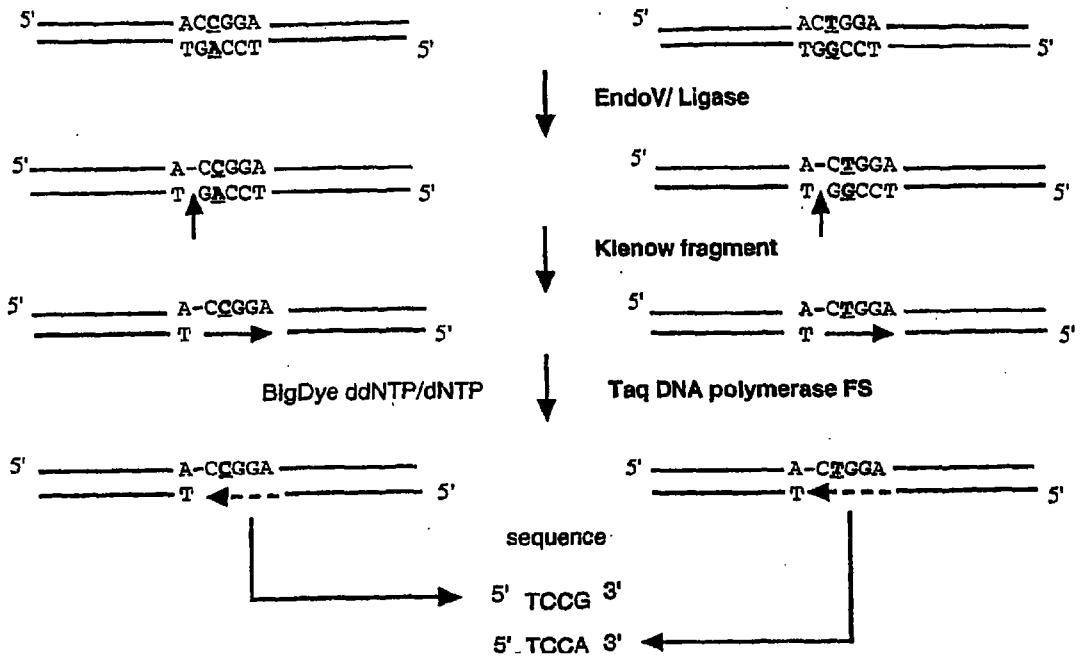


Figure 17


A
p53 R248W(C->T)
5' ACCGGA 5'
TGACCT
5' ACTGGA 5'
TGGCCT
EndoV/ Ligase
5' A-CCGGA 5'
T GACCT
5' A-CTGGA 5'
T GGCCT
Klenow fragment
5' A-CCGGA 5'
T
5' A-CTGGA 5'
T
BigDye ddNTP/dNTP
Taq DNA polymerase FS
5' A-CCGGA 5'
T
5' A-CTGGA 5'
T
sequence
5' TCCG 3'
5' TCCA 3'

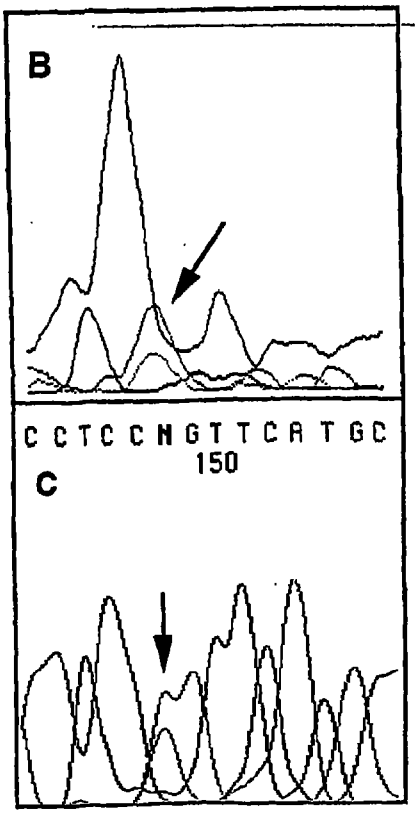

B
C C T C C N G T T C A T G C
150
C

Figure 18

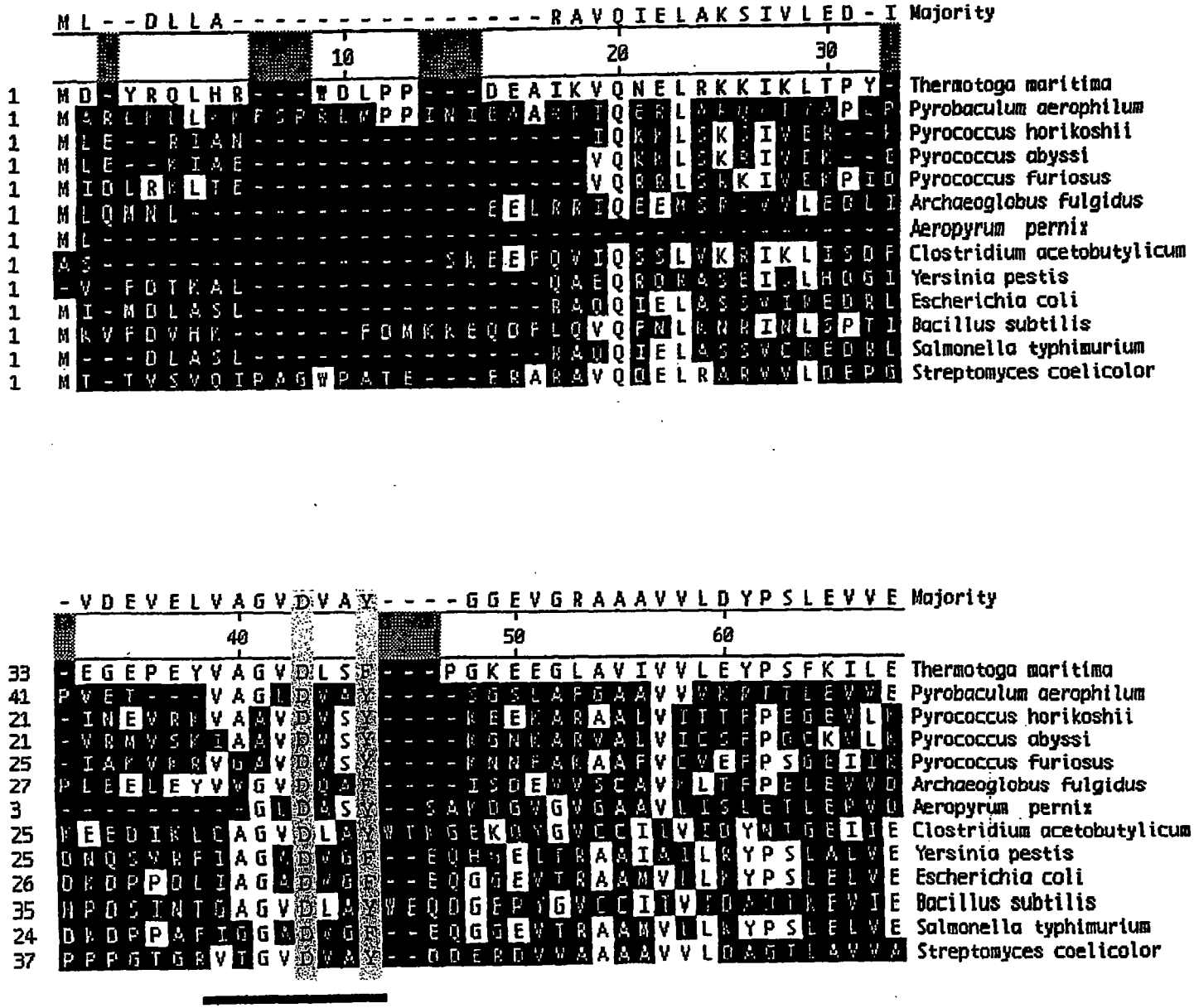
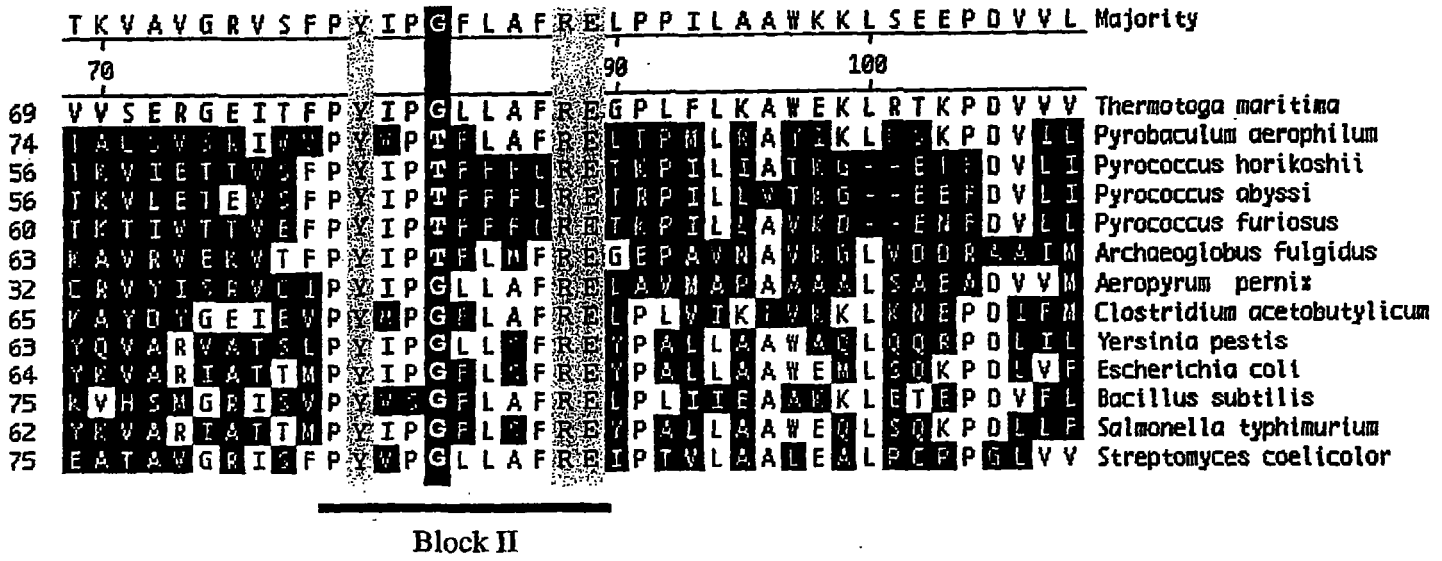

Majority
Thermotoga maritima
Pyrobaculum aerophilum
Pyrococcus horikoshii
Pyrococcus abyssi
Pyrococcus furiosus
Archaeoglobus fulgidus
Aeropyrum pernix
Clostridium acetobutylicum
Yersinia pestis
Escherichia coli
Bacillus subtilis
Salmonella typhimurium
Streptomyces coelicolor
Block I

Figure 19



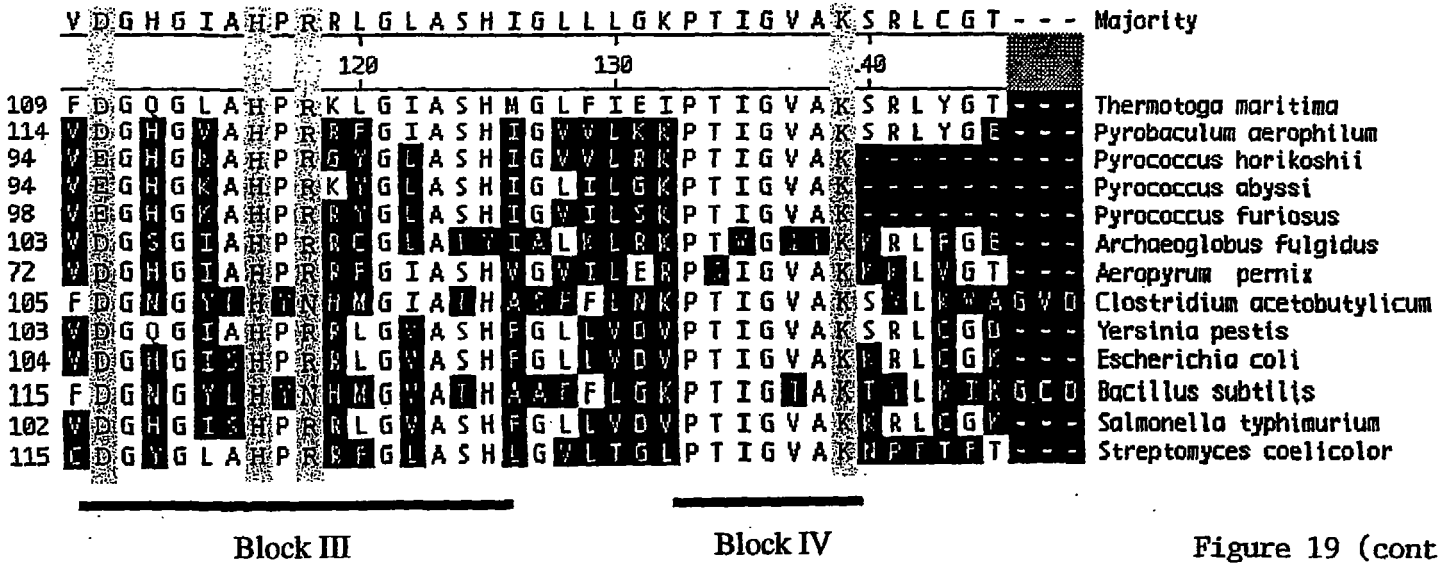
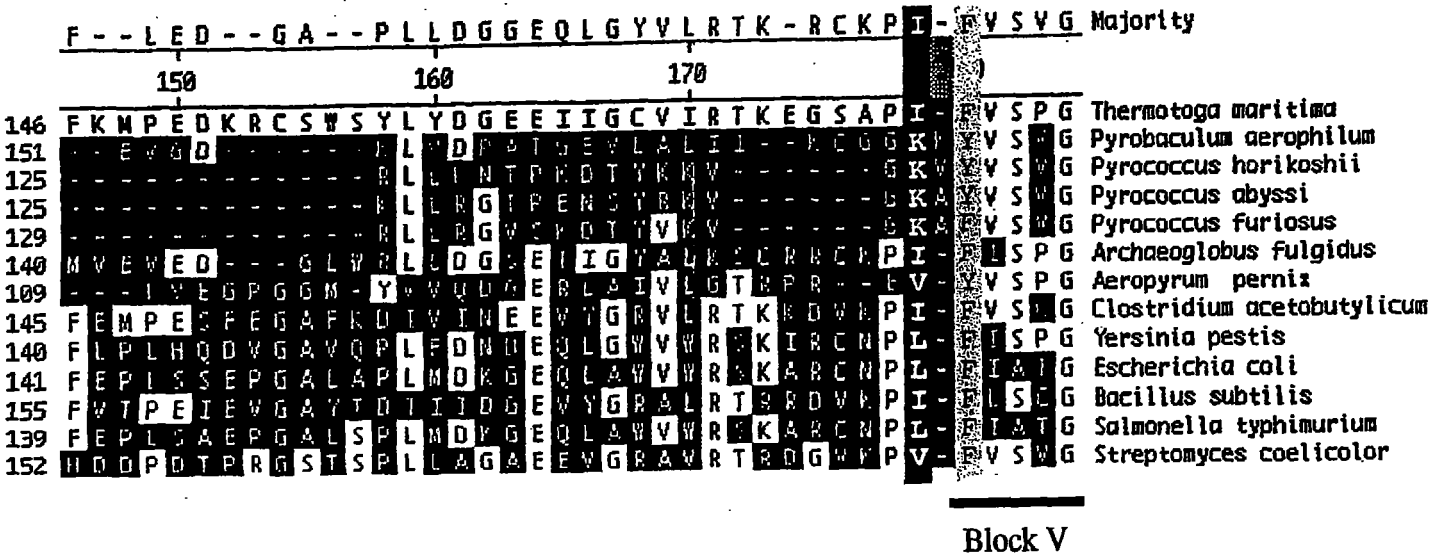


Figure 19 (cont.)



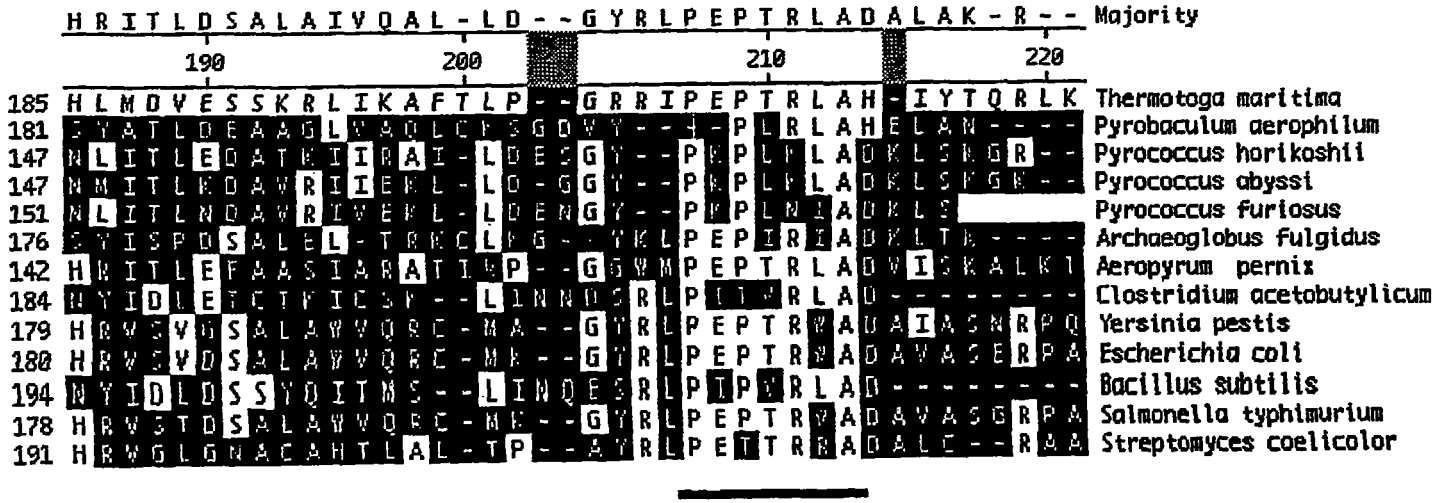


Figure 19 (cont.)

Figure 19 (cont.)

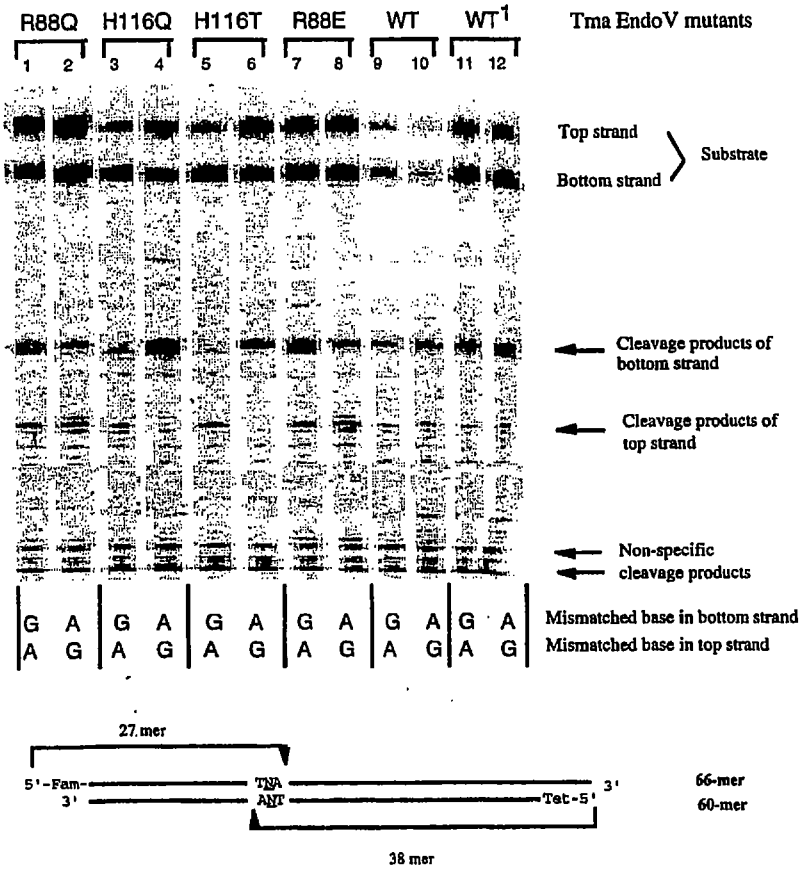

R88Q H116Q H116T R88E WT WT1
Tma EndoV mutants
1 2 3 4 5 6 7 8 9 10 11 12
Top strand
Bottom strand
Substrate
Cleavage products of bottom strand
Cleavage products of top strand
Non-specific cleavage products
G A G A G A G A G A G A
A G A G A G A G A G A G
Mismatched base in bottom strand
Mismatched base in top strand
27 mer
5'-Fam-
3'
TNA
ANT
3'
Tet-5'
66-mer
60-mer
38 mer

Figure 20

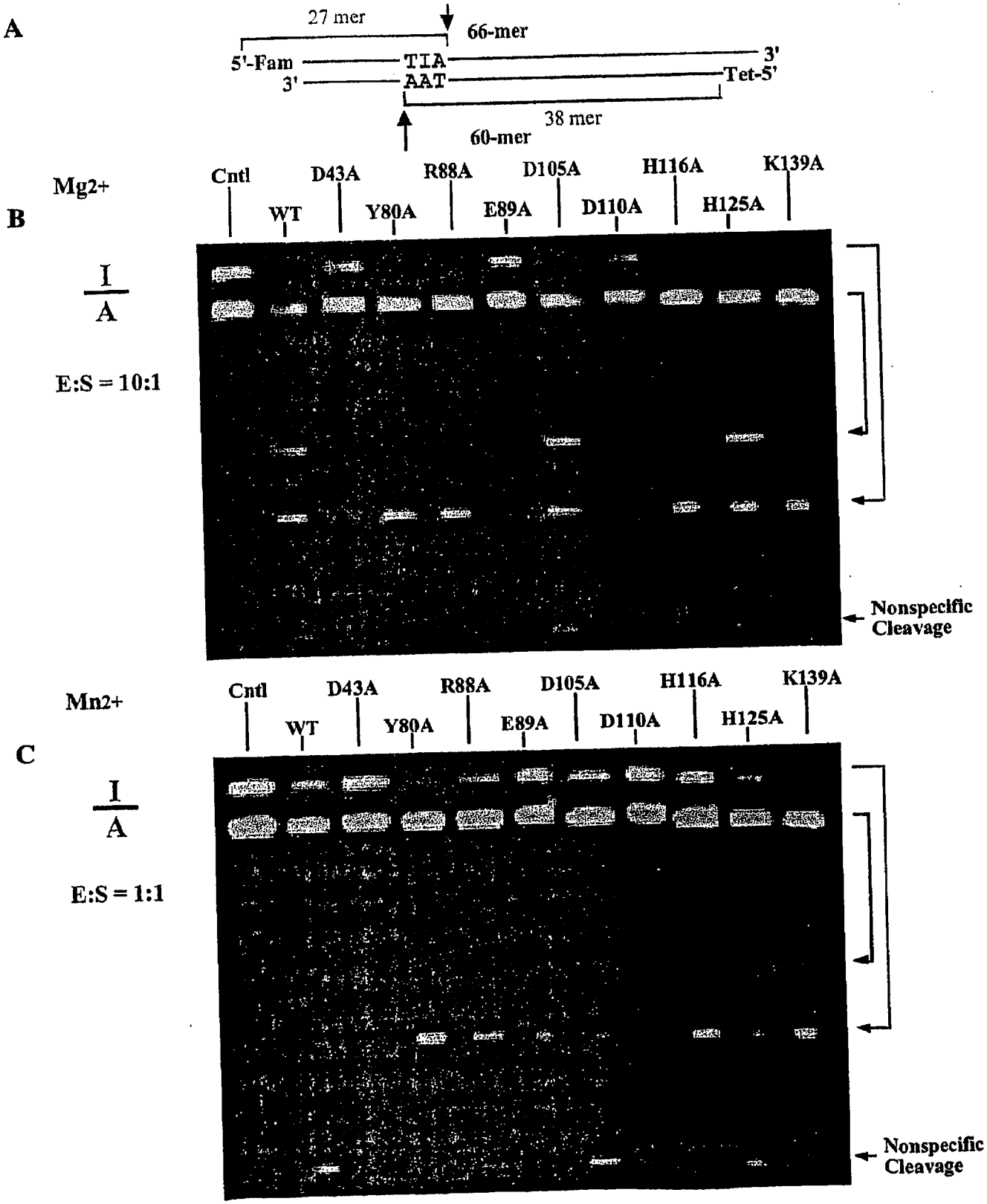

A
27 mer
66-mer
5'-Fam
TIA
3'
3'
AAT
Tet-5'
38 mer
60-mer
B
Mg2+
Cntl
D43A
R88A
D105A
H116A
K139A
WT
Y80A
E89A
D110A
H125A
I
A
E:S = 10:1
Nonspecific Cleavage
C
Mn2+
Cntl
D43A
R88A
D105A
H116A
K139A
WT
Y80A
E89A
D110A
H125A
I
A
E:S = 1:1
Nonspecific Cleavage

Figure 21

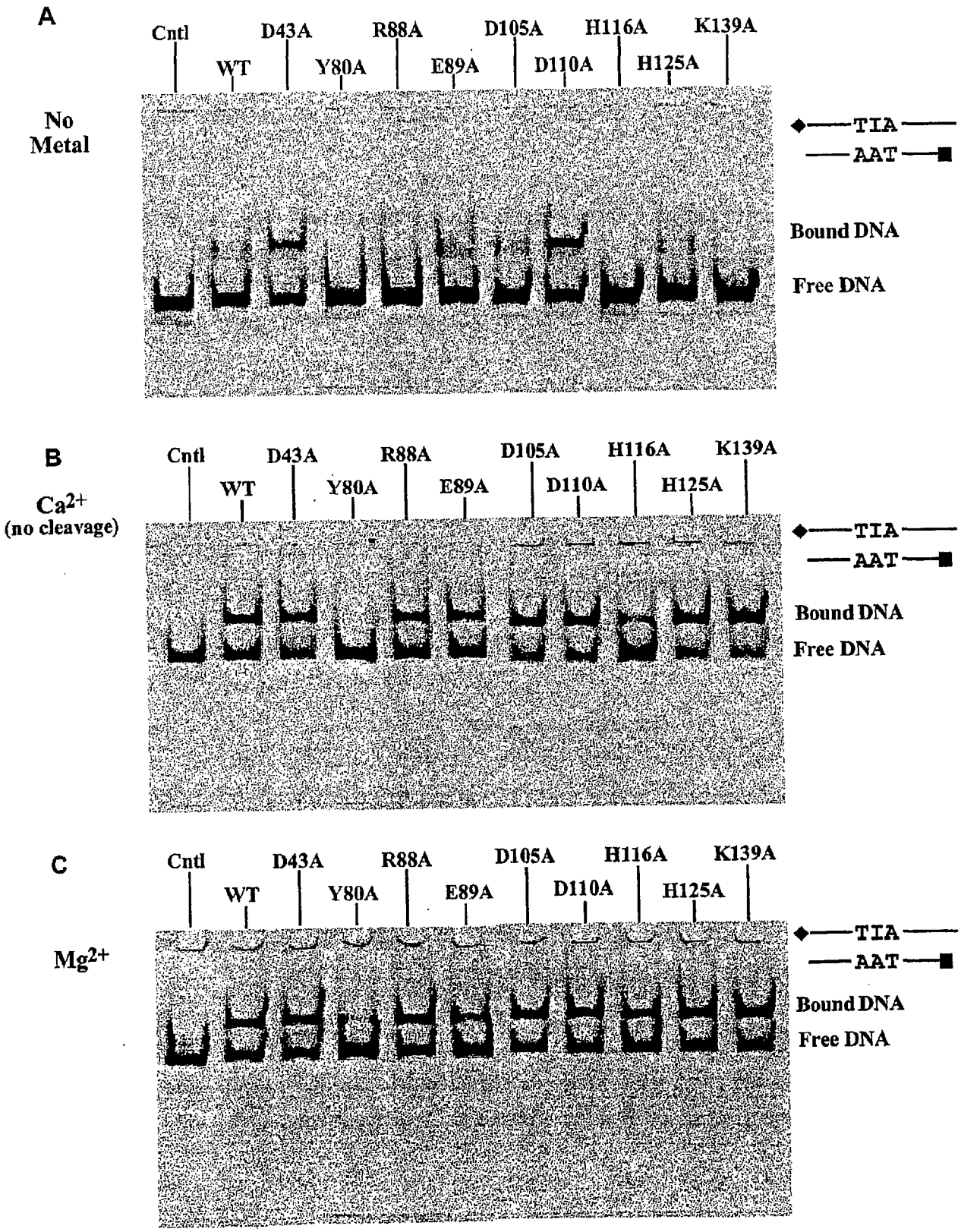

A
Cntl
WT
D43A
Y80A
R88A
E89A
D105A
D110A
H116A
H125A
K139A
No Metal
TIA
AAT
Bound DNA
Free DNA
B
Ca2+ (no cleavage)
C
Mg2+

Figure 22

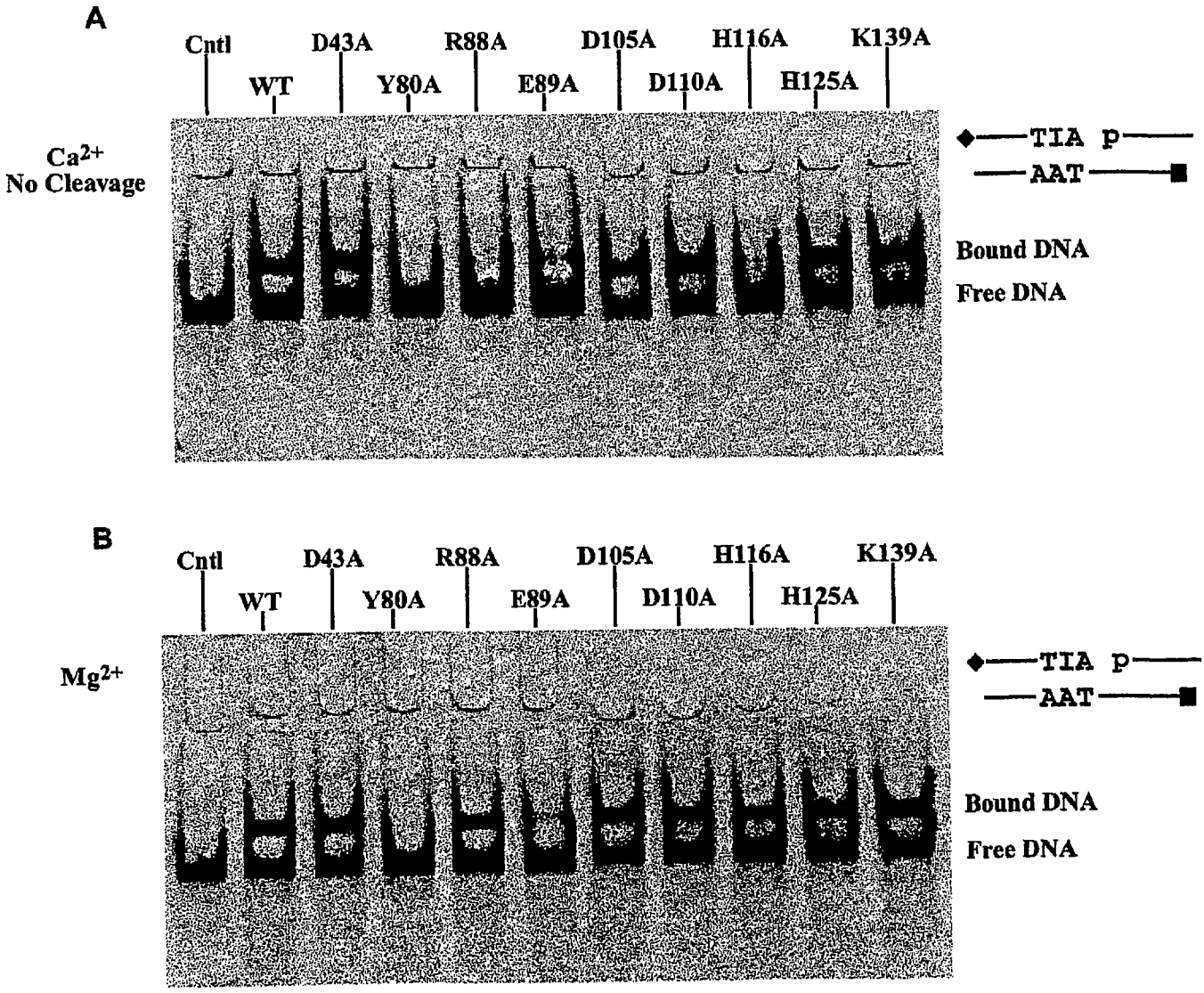

A
Cntl
WT
D43A
Y80A
R88A
E89A
D105A
D110A
H116A
H125A
K139A
Ca2+
No Cleavage
TIA p
AAT
Bound DNA
Free DNA
B
Cntl
WT
D43A
Y80A
R88A
E89A
D105A
D110A
H116A
H125A
K139A
Mg2+
TIA p
AAT
Bound DNA
Free DNA

Figure 23

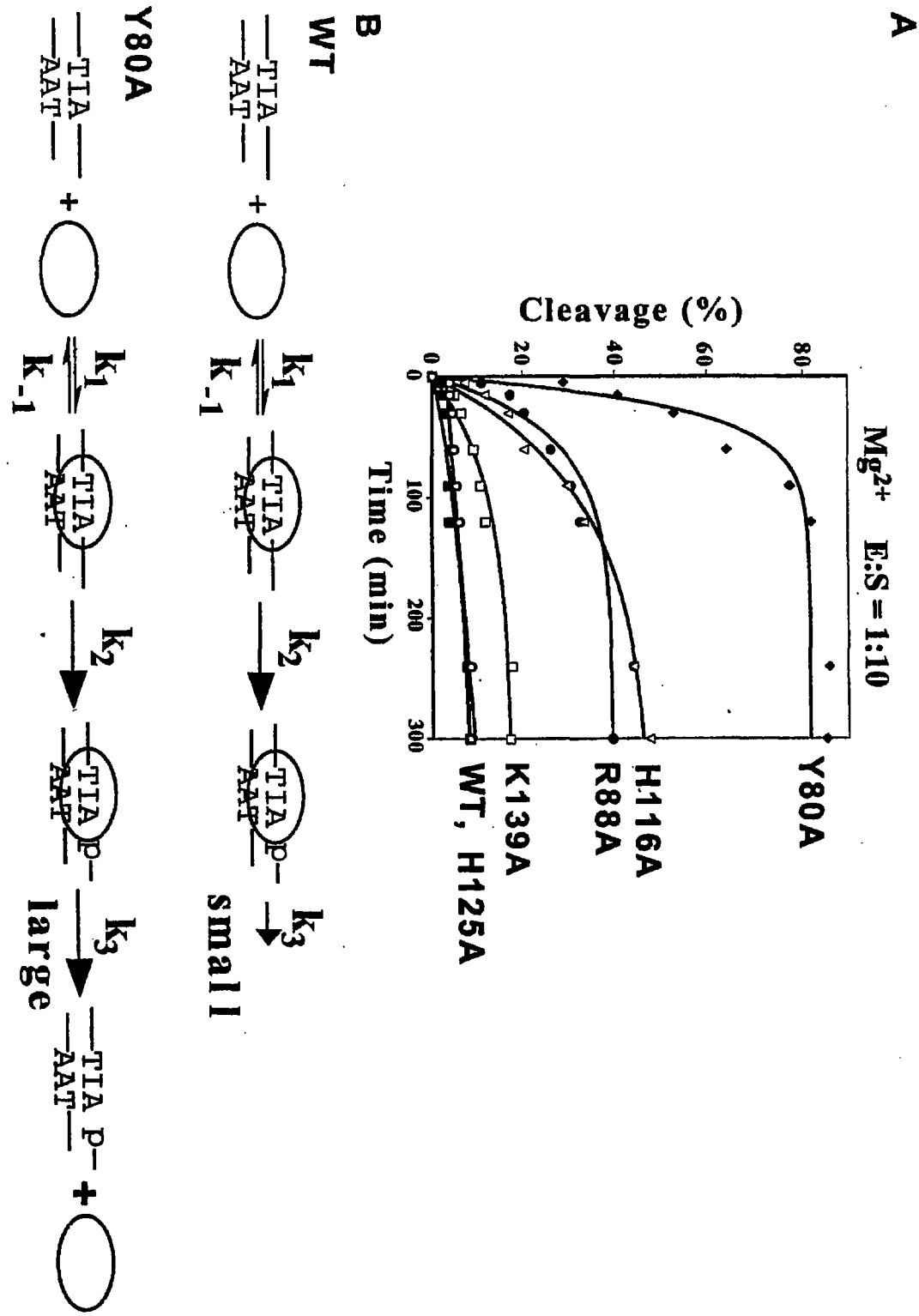

A
Cleavage (%)
0
20
40
60
80
0
100
200
300
Time (min)
Mg2+ E:S = 1:10
Y80A
H116A
R88A
K139A
WT, H125A
B
WT
TIA
AAT
k1
k-1
k2
k3
small
Y80A
large
P

Figure 24

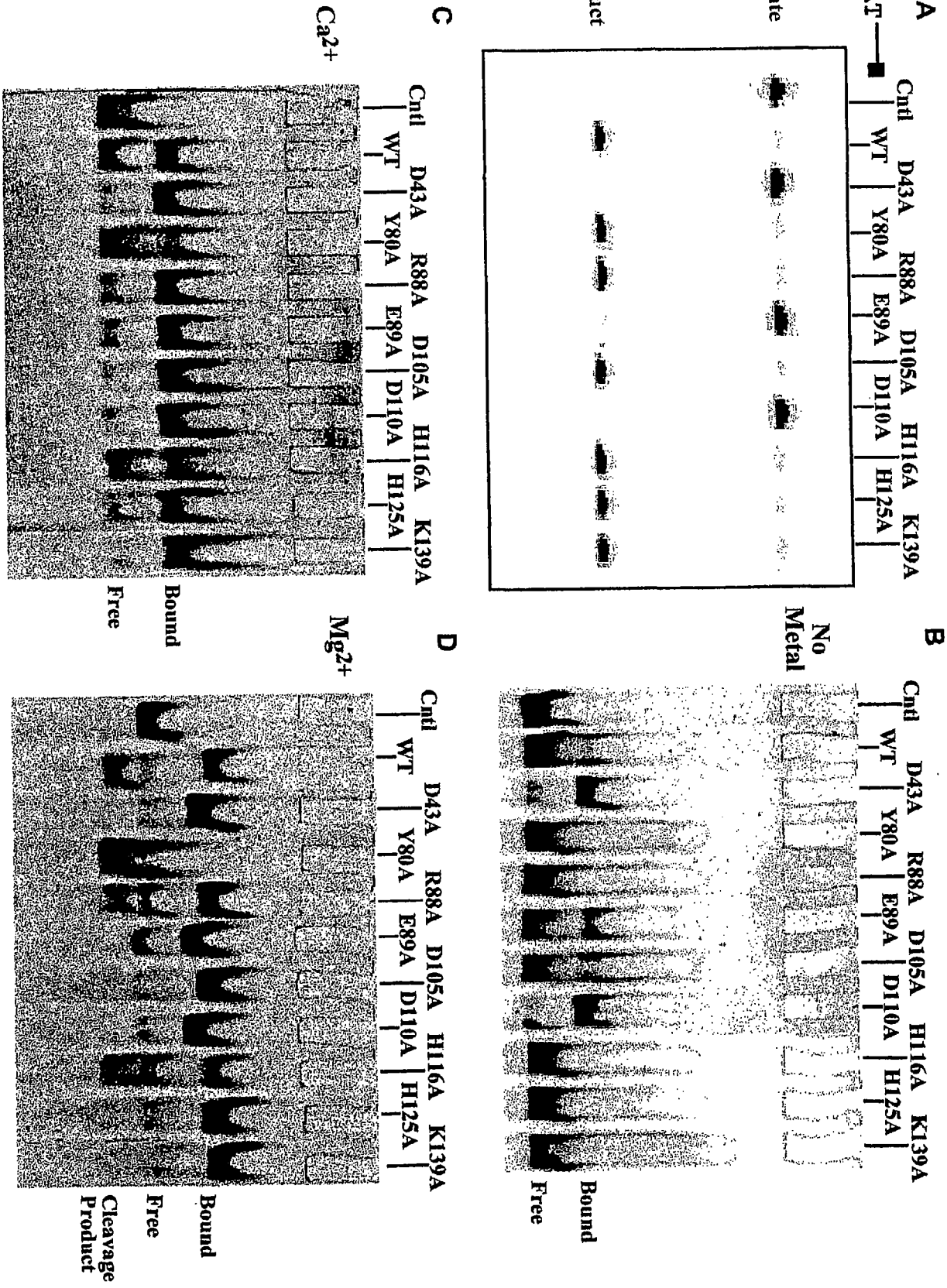

A
AAT
Cntl
WT
D43A
Y80A
R88A
E89A
D105A
D110A
H116A
H125A
K139A
Substrate
Product
B
No Metal
Bound
Free
C
Ca^{2+}
D
Mg^{2+}
Bound
Free
Cleavage Product

Figure 25

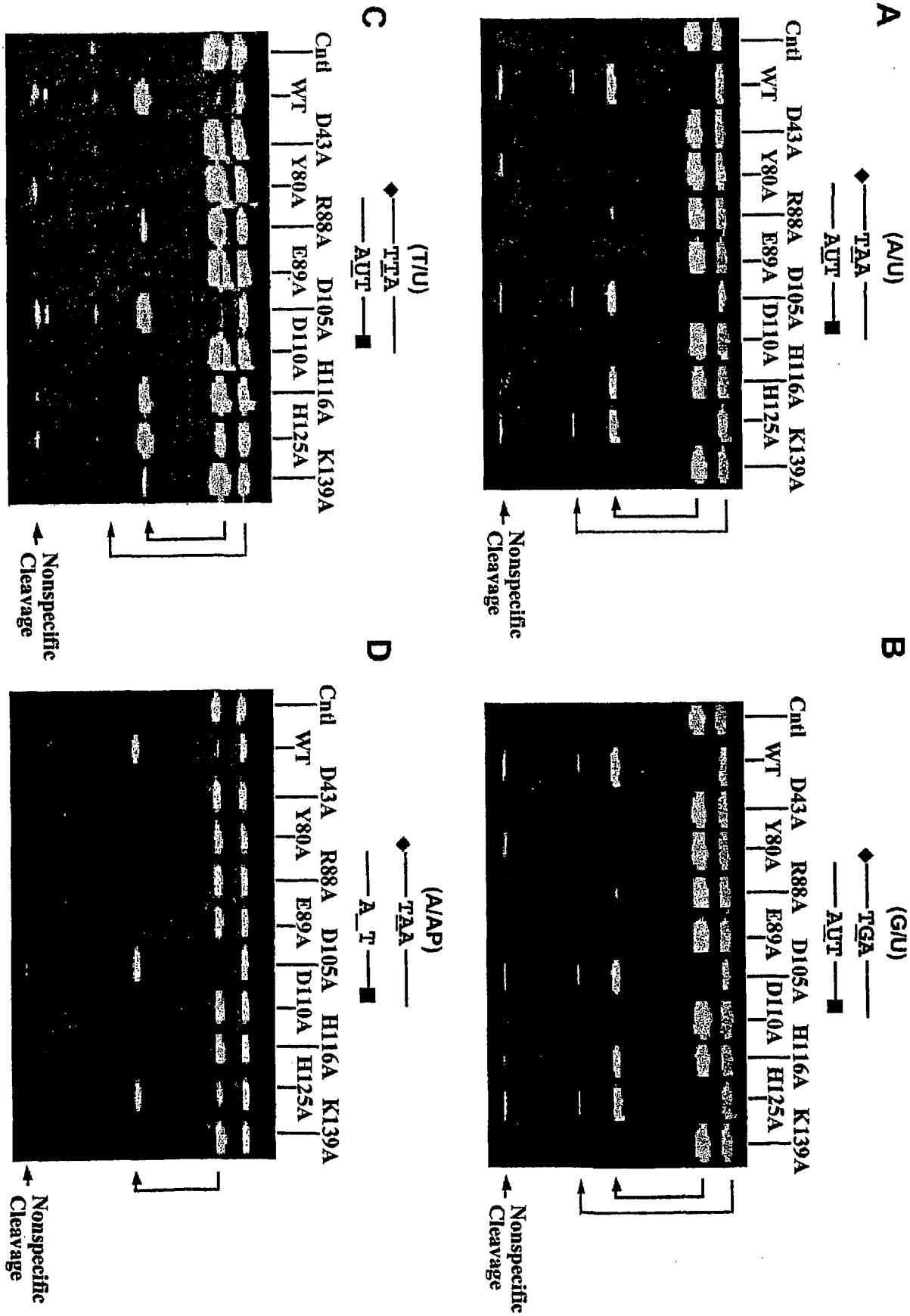

A
(A/U)
TAA
AUT
Cntl
WT
D43A
Y80A
R88A
E89A
D105A
D110A
H116A
H125A
K139A
Nonspecific Cleavage
B
(G/U)
TGA
AUT
Cntl
WT
D43A
Y80A
R88A
E89A
D105A
D110A
H116A
H125A
K139A
Nonspecific Cleavage
C
(T/U)
TTA
AUT
Cntl
WT
D43A
Y80A
R88A
E89A
D105A
D110A
H116A
H125A
K139A
Nonspecific Cleavage
D
(A/AP)
TAA
A_T
Cntl
WT
D43A
Y80A
R88A
E89A
D105A
D110A
H116A
H125A
K139A
Nonspecific Cleavage

Figure 26