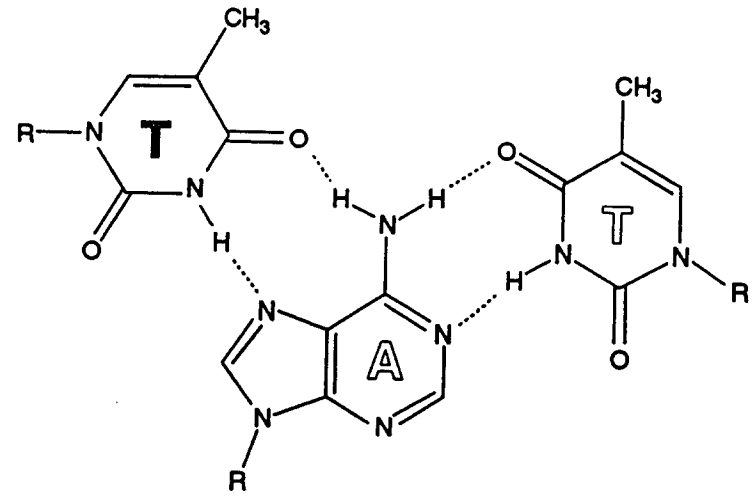
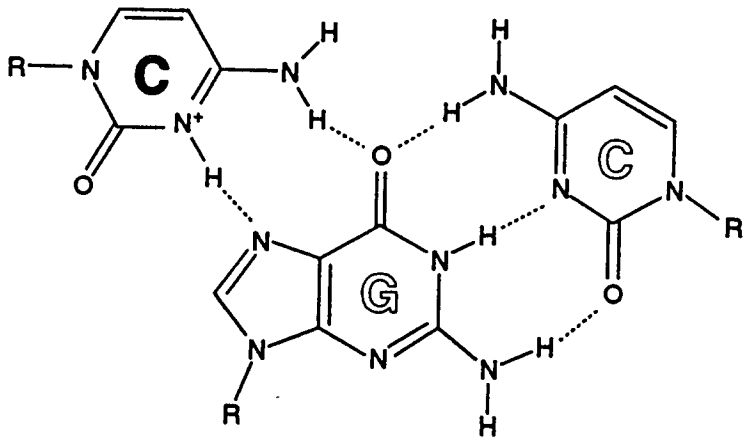
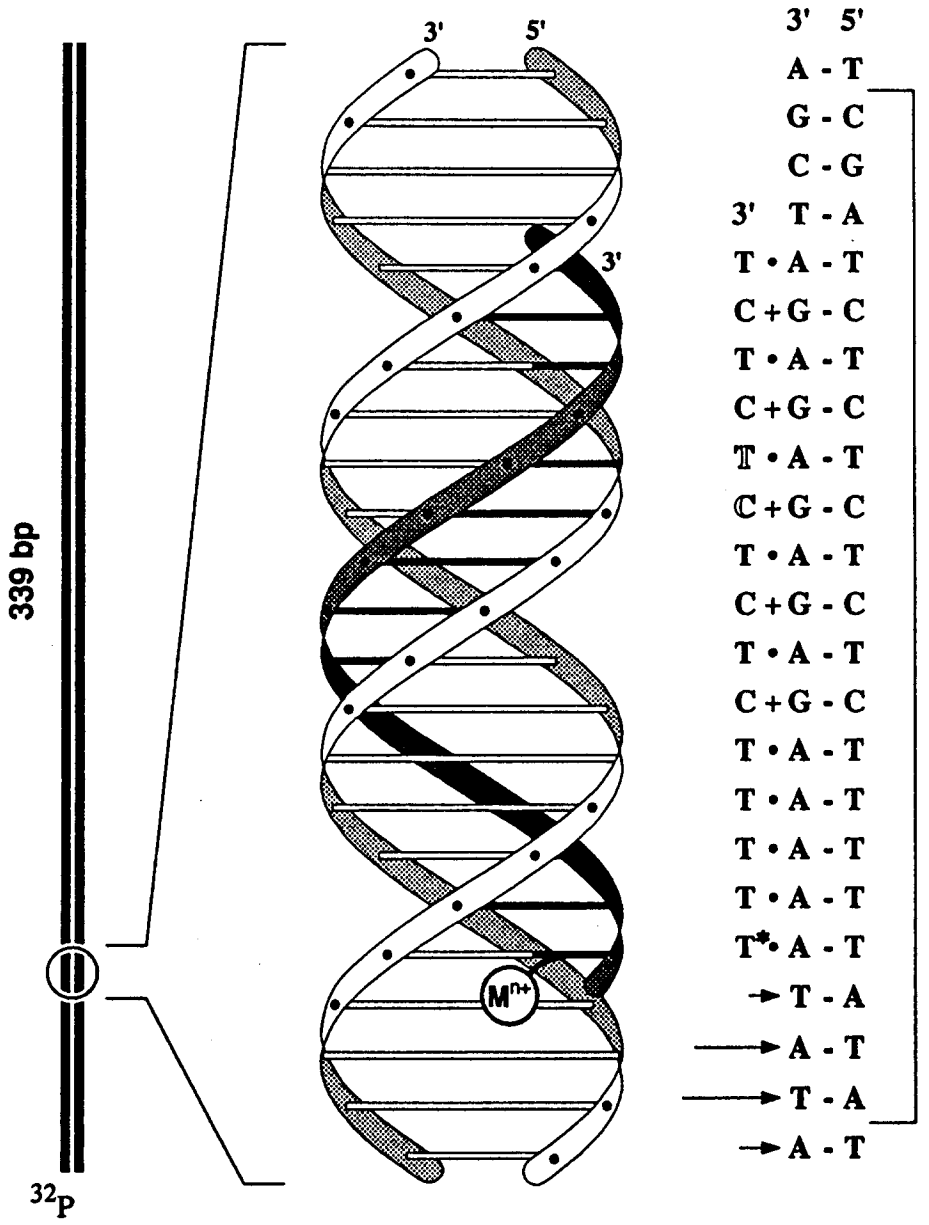
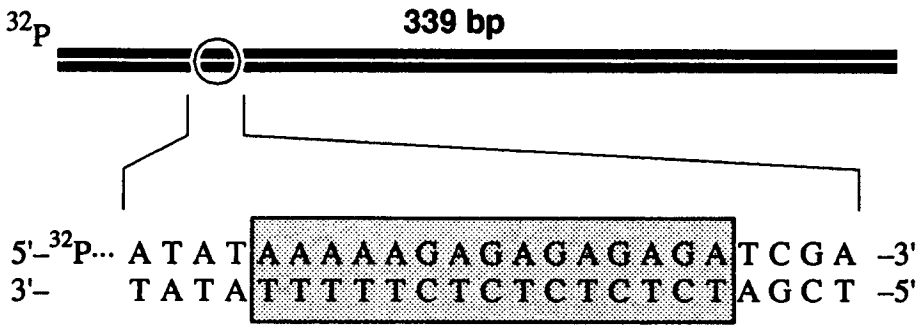
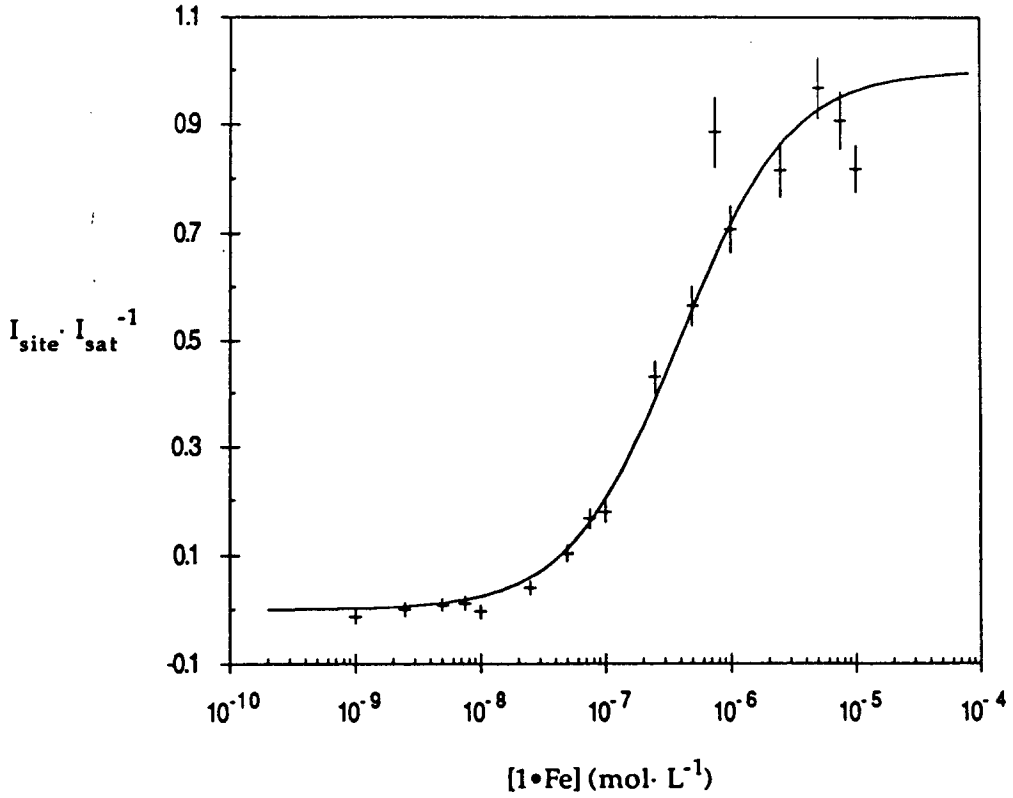

CH3
R
N
T
O
N
H
O
H
N
H
O
CH3
T
N
H
N
R
N
A
N
N
R

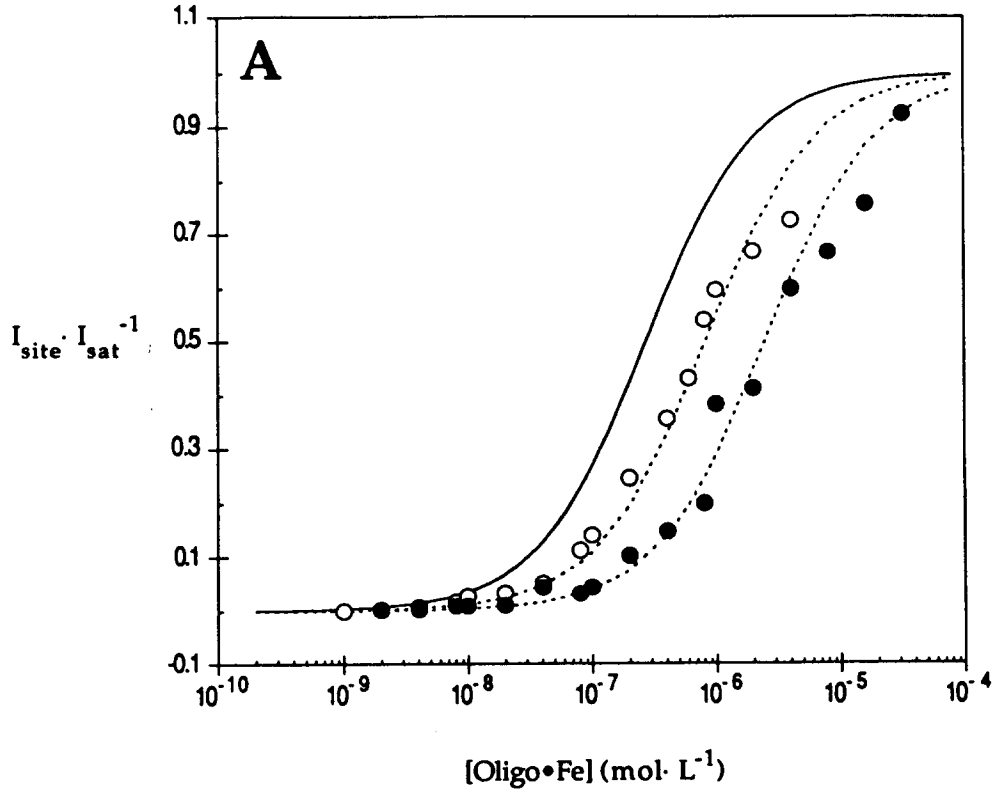

R
N
C
N+
H
O
H
N
H
H
O
H
N
C
N
R
N
G
N
H
N
H
O
N
H
R
H


339 bp
$^{32}P$
3'
5'
3'
$M^{n+}$
3' 5'
A - T
G - C
C - G
3' T - A
T • A - T
C + G - C
T • A - T
C + G - C
T • A - T
C + G - C
T • A - T
C + G - C
T • A - T
T • A - T
T • A - T
T • A - T
T* • A - T
→ T - A
→ A - T
→ T - A
→ A - T




| | |
|---|---|
| **1** | 5'–*T T T T T C T C T C T C T C T |
| **2** | 5'–*T T T T T C T C T C T C T |
| **3** | 5'–*T T T T T C T C T C T |
| **4** | 5'–*T T T T T C T C T C C C T C T |
| **5** | 5'–*T T T T T C T C T T T C T C T |
| **6** | 5'– T T T T T C T C T C T C T C T |


1.1
0.9
0.7
0.5
0.3
0.1
-0.1
$I_{site} \cdot I_{sat}^{-1}$
$10^{-10}$
$10^{-9}$
$10^{-8}$
$10^{-7}$
$10^{-6}$
$10^{-5}$
$10^{-4}$
[1•Fe] (mol· $L^{-1}$)


A
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
$I_{site} \cdot I_{sat}^{-1}$
$10^{-10}$
$10^{-9}$
$10^{-8}$
$10^{-7}$
$10^{-6}$
$10^{-5}$
$10^{-4}$
[Oligo•Fe] (mol· $L^{-1}$)

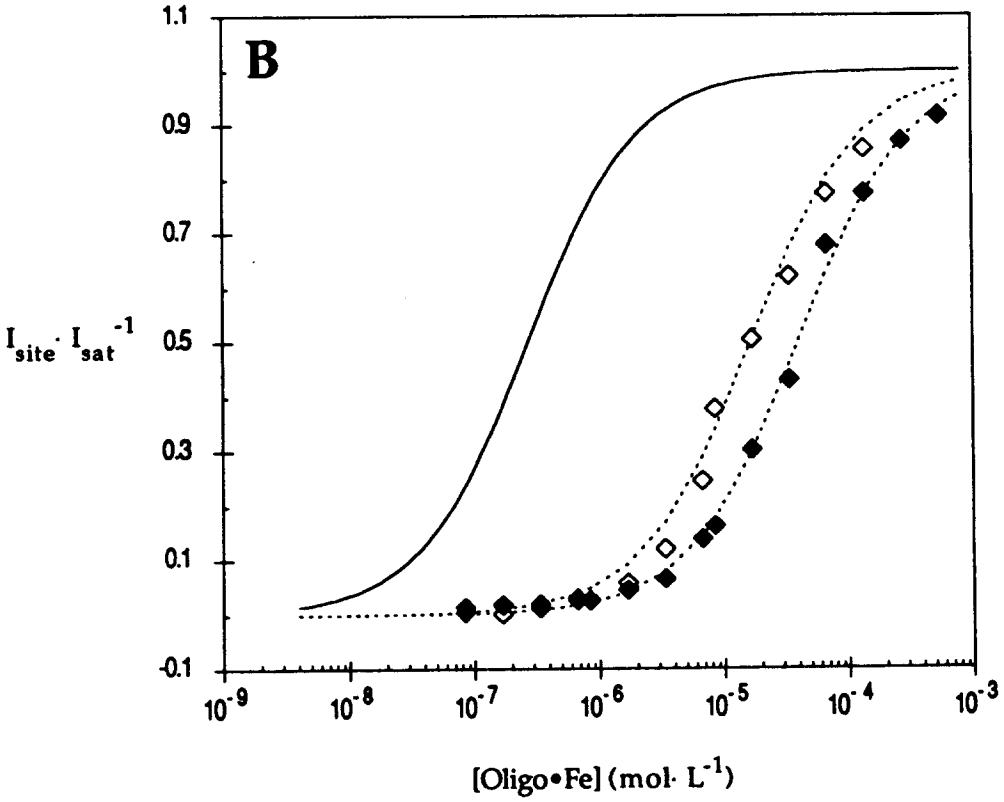
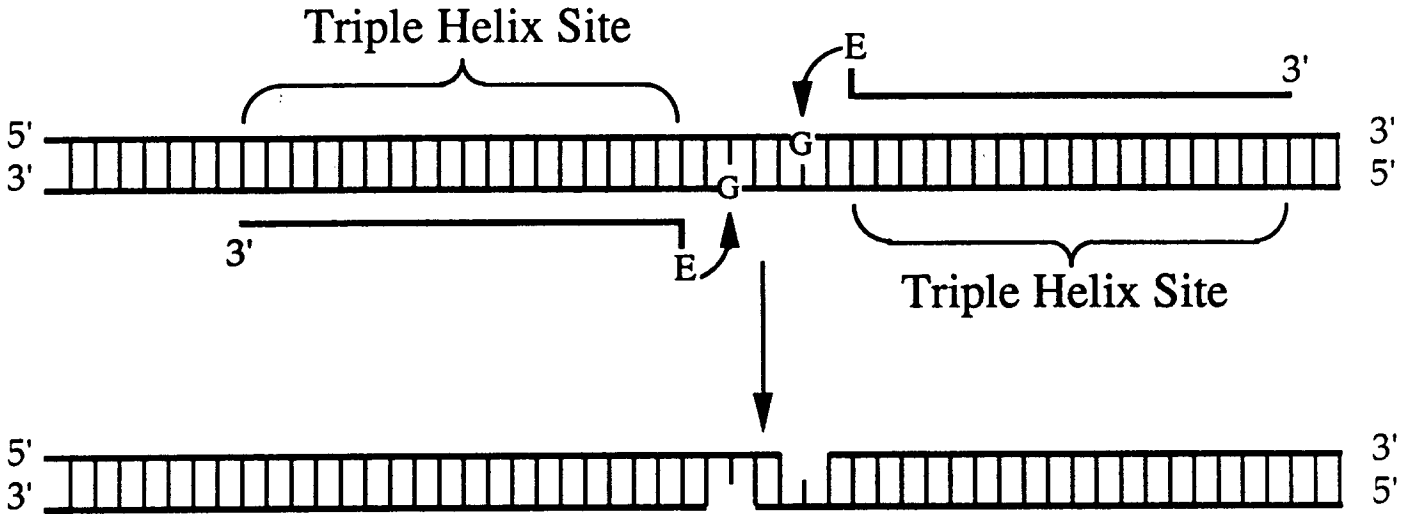
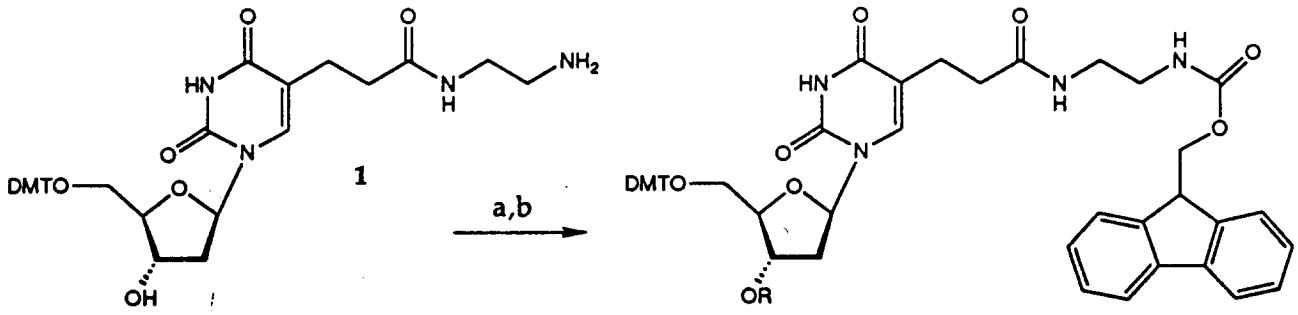

B
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
$I_{site} \cdot I_{sat}^{-1}$
$10^{-9}$
$10^{-8}$
$10^{-7}$
$10^{-6}$
$10^{-5}$
$10^{-4}$
$10^{-3}$
[Oligo•Fe] (mol· $L^{-1}$)

**Table II.** Length and Sequence Mismatch Effects on $K_T$ at pH 7.0 and 24 °C.[a]

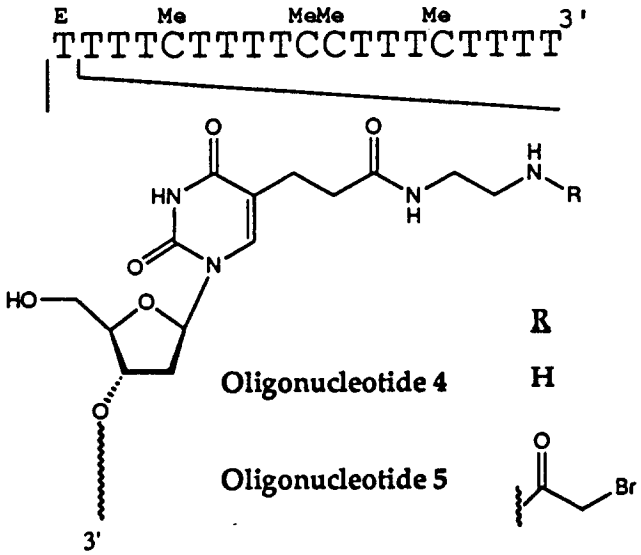
| Oligo-nucleotide | Length (nts) | No. T's (nts) | No. C's (nts) | Base Mismatch | $K_T$ ($M^{-1}$) | $\Delta G_T$ (kcal·mol$^{-1}$) |
|---|---|---|---|---|---|---|
| 1 | 15 | 10 | 5 | - | 3.7 (±1.1) x $10^6$ | −9.0 (±0.2) |
| 2 | 13 | 9 | 4 | - | 1.8 (±0.4) x $10^6$ | −8.5 (±0.1) |
| 3 | 11 | 8 | 3 | - | 6.2 (±3.8) x $10^5$ | −7.9 (±0.6) |
| 4 | 15 | 9 | 6 | C○AT | 6.1 (±2.3) x $10^4$ | −6.5 (±0.2) |
| 5 | 15 | 11 | 4 | T○GC | 2.5 (±0.1) x $10^4$ | −6.0 (±0.1) |

[a] Values reported in the table are mean values measured from affinity cleavage titration experiments performed in Association buffer (100 mM $Na^+$, 1 mM spermine tetrahydrochloride, 50 mM Tris-acetate, pH 7.0).

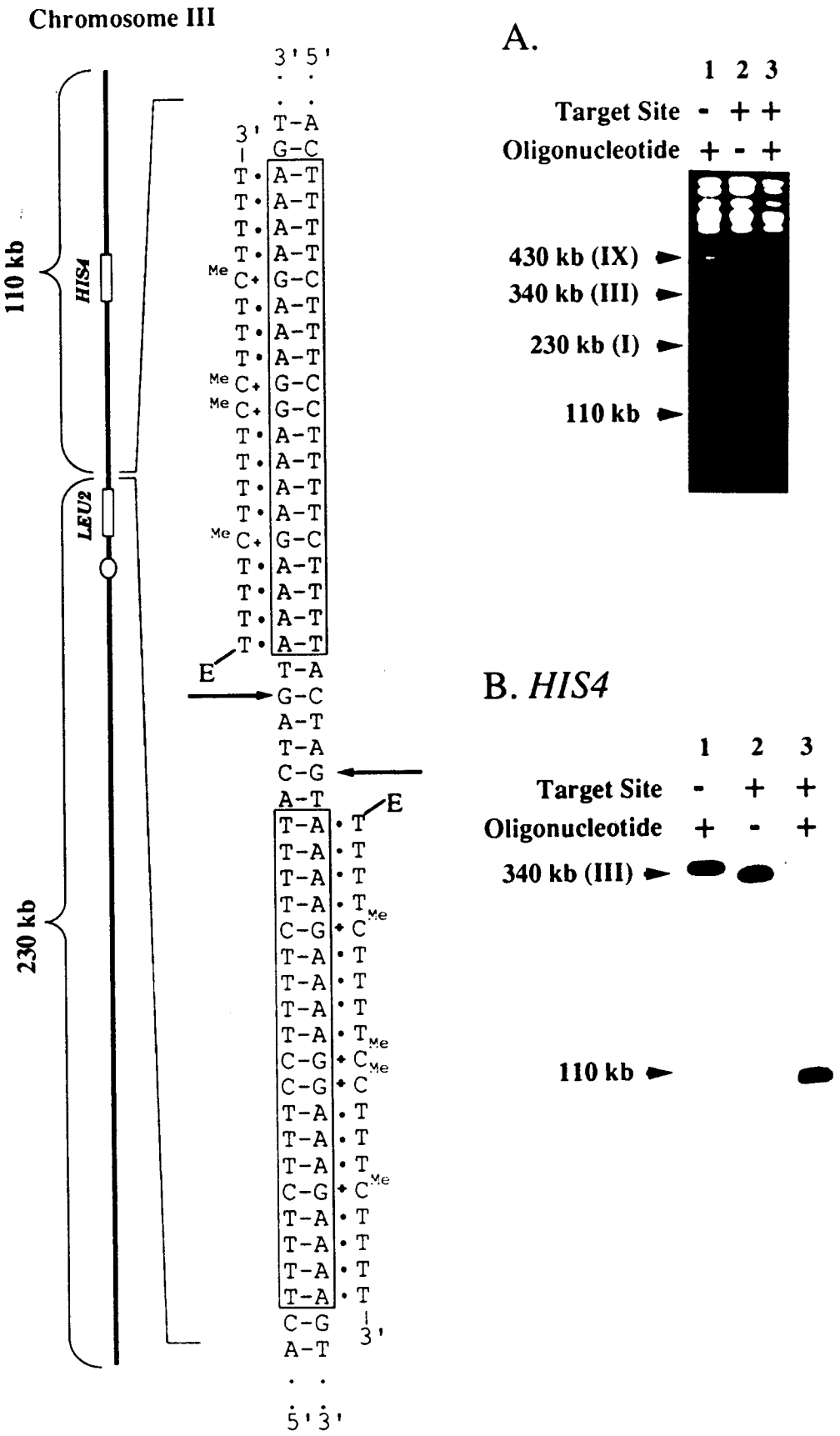

Triple Helix Site
E
3'
5'
3'
G
G
3'
3'
E
Triple Helix Site
5'
3'
3'
5'

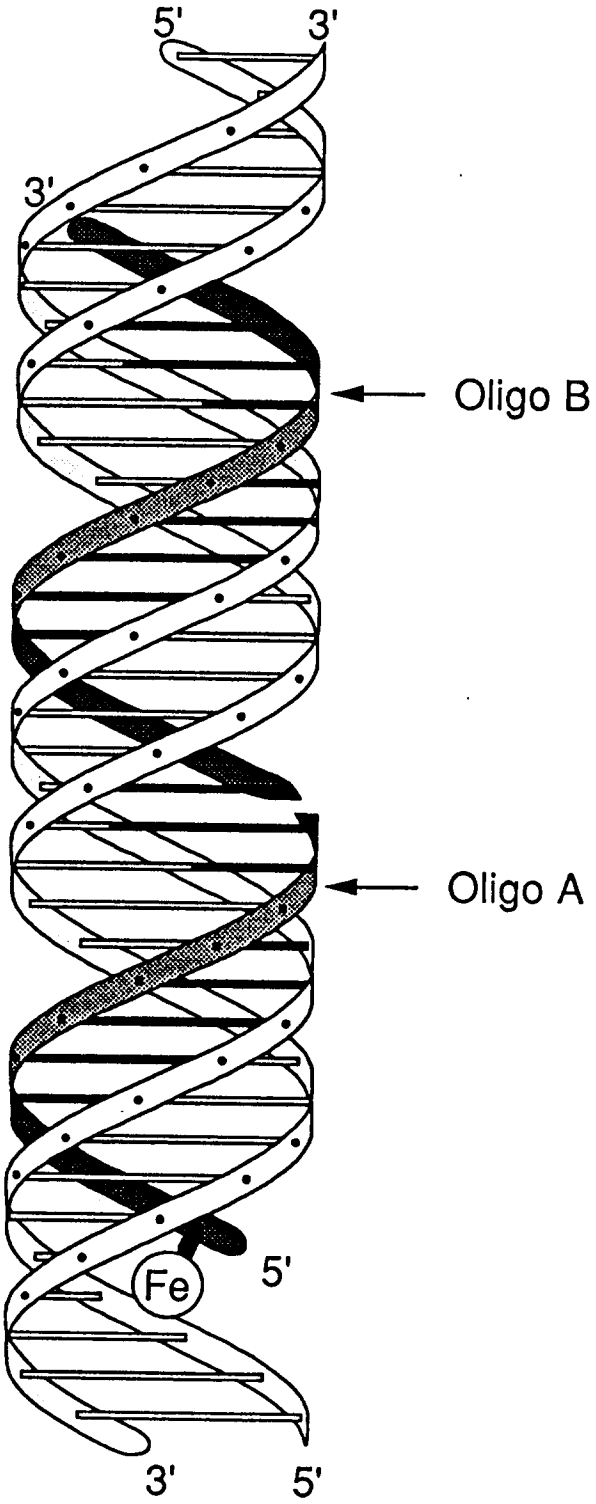


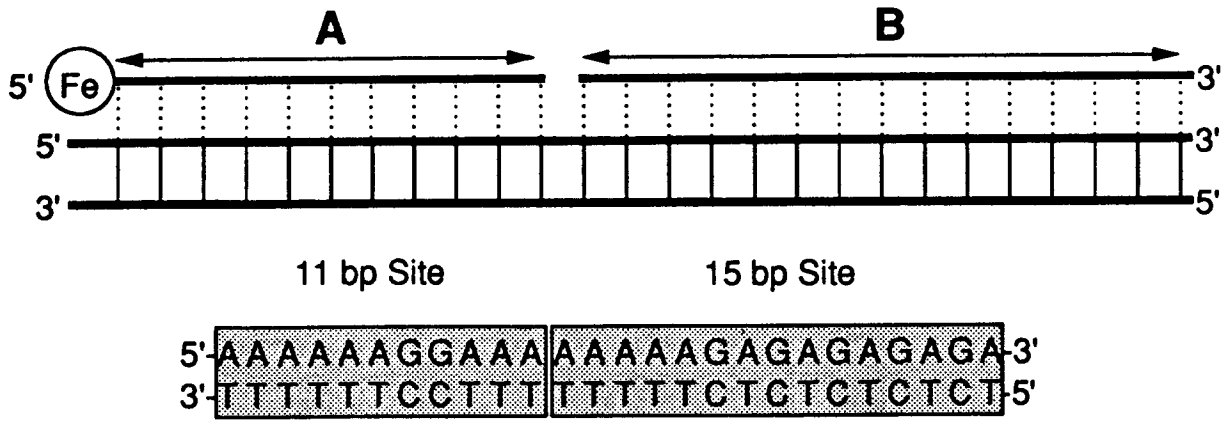

| R | |
|---|---|
| 2 | H |
| 3 | $P[N(iPr)_2]OCH_2CH_2CN$ |




| R | |
|---|---|
| Oligonucleotide 4 | H |
| Oligonucleotide 5 |  |


Chromosome III
110 kb
230 kb
HIS4
LEU2
E
E
A.
1 2 3
Target Site - + +
Oligonucleotide + - +
430 kb (IX)
340 kb (III)
230 kb (I)
110 kb
B. HIS4
1 2 3
Target Site - + +
Oligonucleotide + - +
340 kb (III)
110 kb


5'
3'
3'
Oligo B
Oligo A
Fe
5'
3'
5'




**1** 5'-*TTTTTCCTTT **(A)**

**2** 5'-*TTTTTCTCTCTCTCT **(B)**

# *Quantitative Affinity Cleavage Reaction Conditions*

25 mM TRISOAc, pH 7.0; 10 mM NaCl; 1mM Spermine;

100 μM bp CT DNA; 4 mM DTT; ~10,000 cpm Target Site.

24 hr Room Temperature Preincubation;

6 hr Cleaving Reactions.

**Oligonucleotide Concentrations used:**

**11mer:** 100 pM → 20 μM;

**15mer:** 1 μM.

# *Binding Constants Obtained from Quantitative Affinity Cleavage Experiments: 11mer + 15mer*

11mer alone:

$K_a = 1.16 (\pm 0.70) \cdot 10^6\ M^{-1}$; $K_d = 862$ nM

15mer alone:

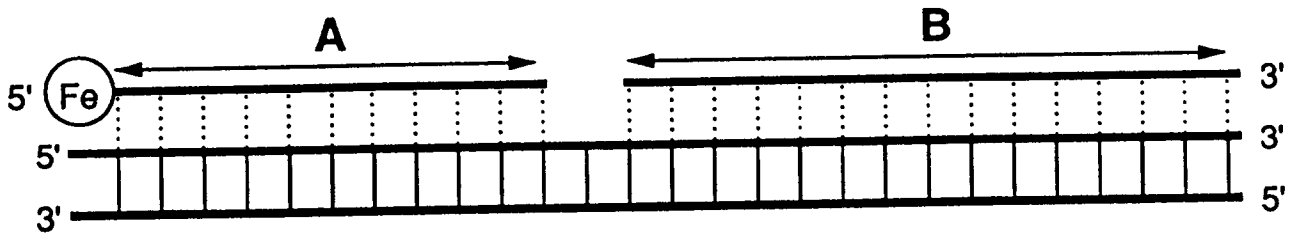
$K_a = 1.26 (\pm 0.52) \cdot 10^7\ M^{-1}$; $K_d = 79$ nM

11mer + 15mer (1 μM):

$K_d = 2.32 (\pm 1.14) \cdot 10^7\ M^{-1}$; $K_d = 43$ nM

$\Delta\Delta G = 1.76$ kcal/mol

$\therefore$ 20-fold Enhancement

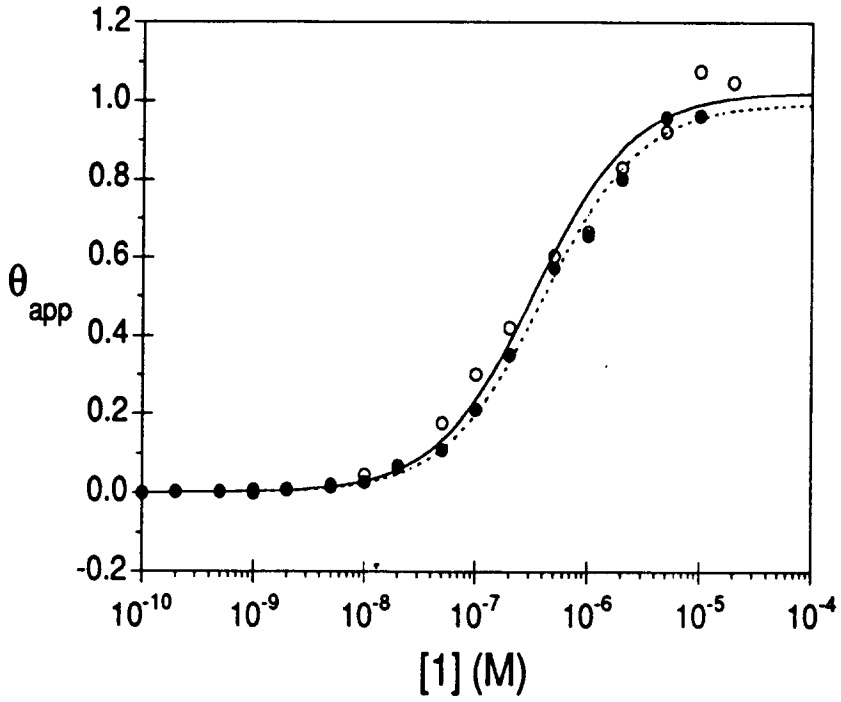



11 bp Site 15 bp Site

5'-AAAAAAGGAAAAAAAAAAGAGAGAGAGA-3'
3'-TTTTTTCCTTTTTTTTTCTCTCTCT-5'

1 5'-*TTTTTCCTTT (A)

2 5'-*TTTTTCTCTCTCTCT (B)


1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
θ app
10-10
10-9
10-8
10-7
10-6
10-5
10-4
[1] (M)

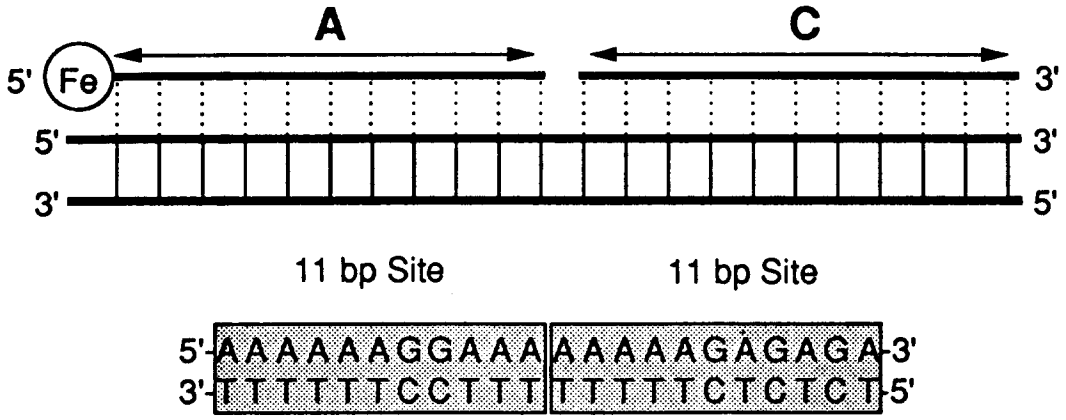
# *Binding Constants Obtained from Quantitative Affinity Cleavage Experiments: 11mer + 15mer in the Presence of a One Base Pair Gap*

11mer alone:

Ka = 2.89 (± 0.35) • $10^6$ $M^{-1}$; Kd = 346 nM

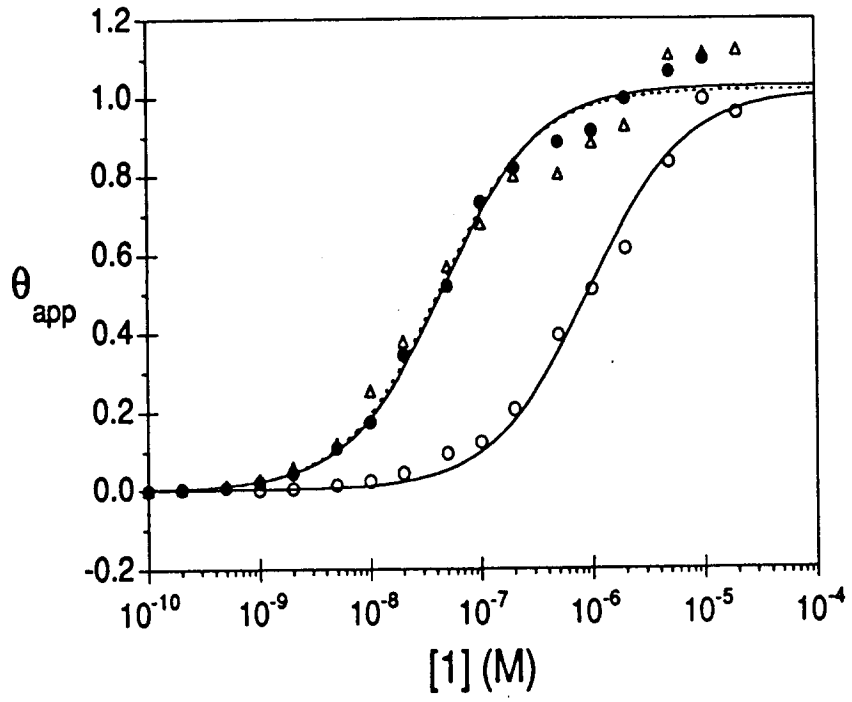
11mer + 15mer (1 μM):

Kd = 2.51 (± 0.51) • $10^6$ $M^{-1}$; Kd = 398 nM




**1** 5'-*TTTTTCCTTT **(A)**

**3** 5'-*TTTTTCTCTCT **(C)**


1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
θ app
10-10
10-9
10-8
10-7
10-6
10-5
10-4
[1] (M)

# *Binding Constants obtained from Quantitative Affinity Cleavage Reactions: 11mer + 11mer*

11mer alone:

Ka = 1.16 (± 0.70) • $10^6$ $M^{-1}$; Kd = 862 nM

11mer + 15mer:

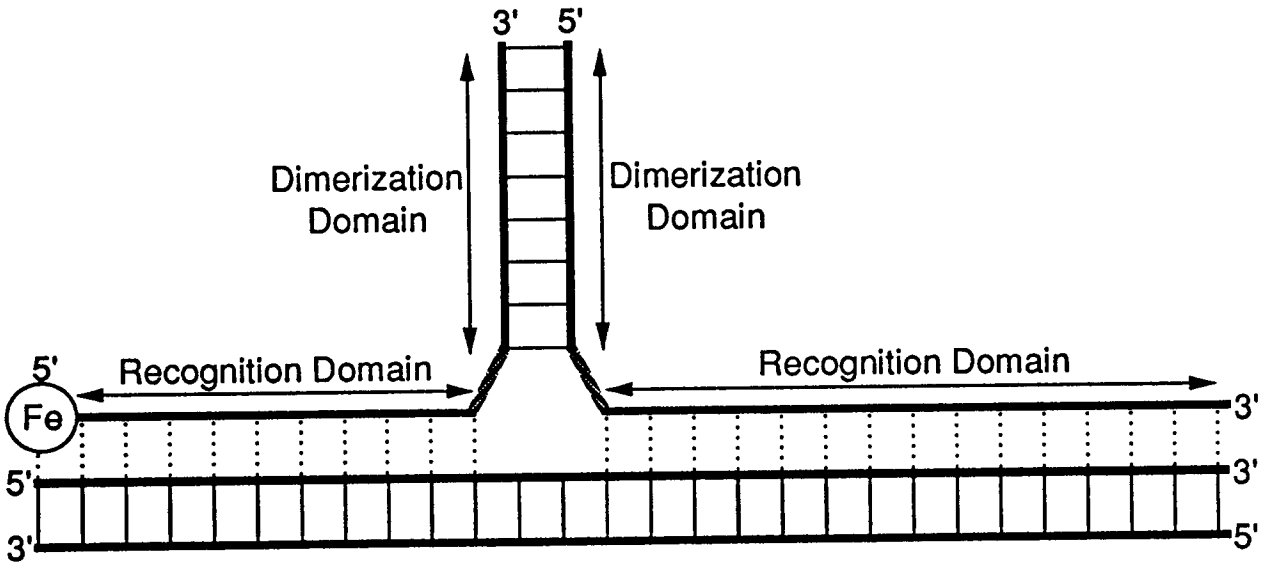
Kd = 2.32 (± 1.14) • $10^7$ $M^{-1}$; Kd = 43 nM

11mer + 11mer:

Kd = 2.37 (± 0.33) • $10^7$ $M^{-1}$; Kd = 42 nM

$\Delta\Delta G$ = 1.76 kcal/mol

$\therefore$ still 20-fold Enhancement



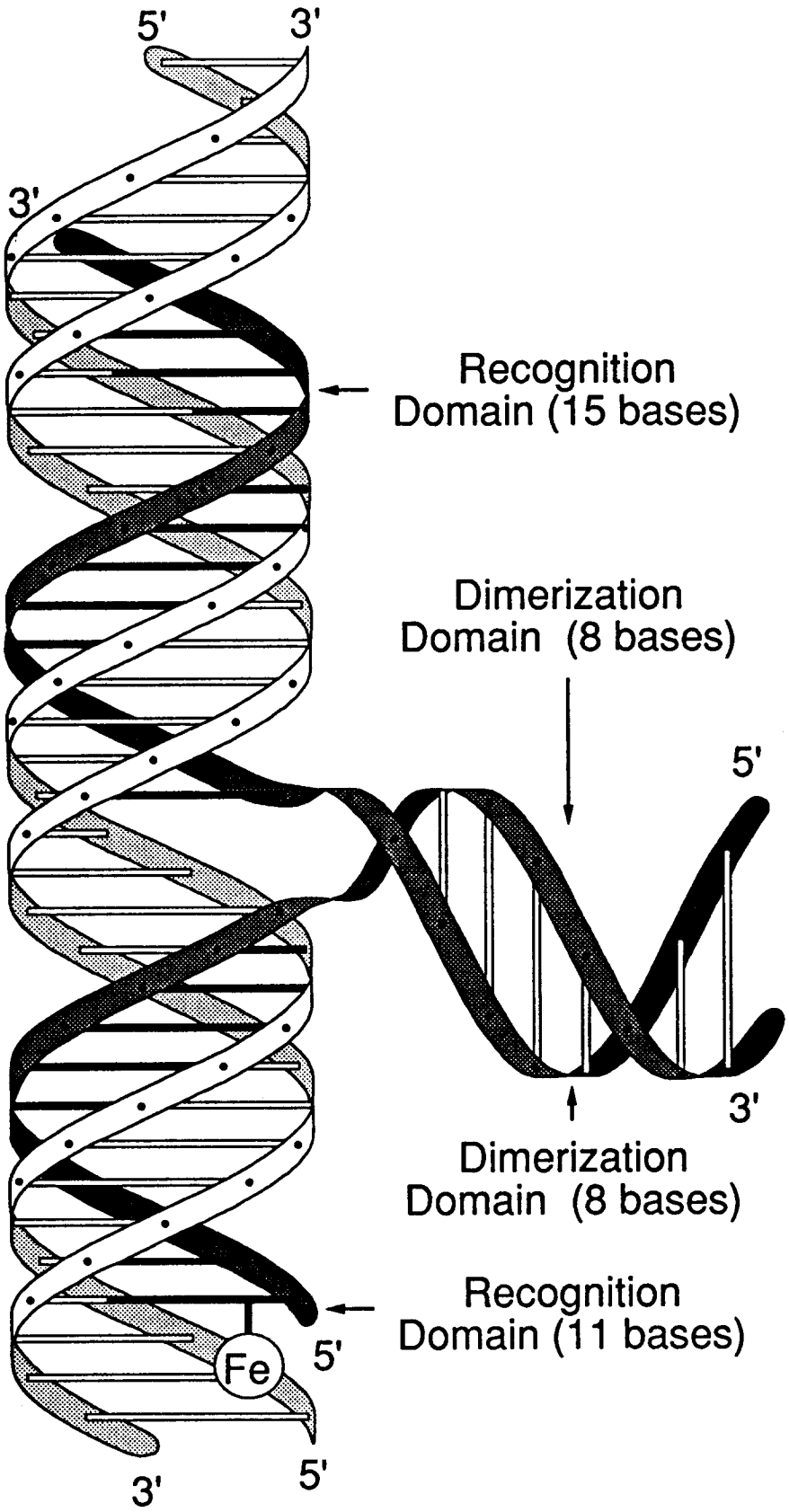

11 bp Site 15 bp Site

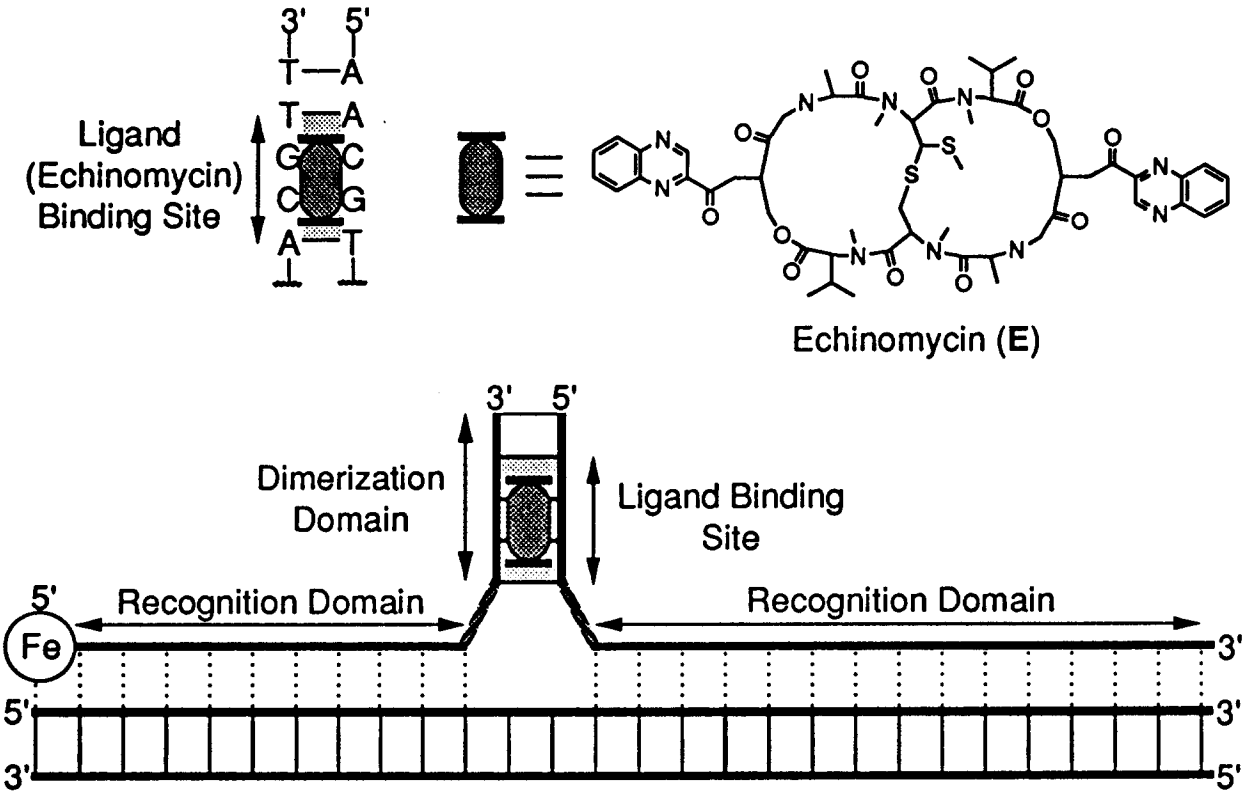
5'-AAAAAAGGAAAAAAAAAAGAGAGAGAGAGA-3'
3'-TTTTTTCCTTTTTTTTTCTCTCTCTCTCT-5'

**1** 5'-*TTTTTTTTCCTTTCGTCAATCG **(11 bp Site, T*)**

**2** 5'-CGATTGACCTTTTTCTCTCTCTCT **(15 bp Site, No T*)**

**3** 5'-CGATTGACCTTTTTCTCTCTCTCT **(15 bp Site, T*)**


5'
3'
3'
Recognition
Domain (15 bases)
Dimerization
Domain (8 bases)
5'
3'
Dimerization
Domain (8 bases)
Recognition
Domain (11 bases)
Fe
5'
3'
5'




5'-AAAAAAGGAAAA A AAAAAAGAGAGAGAGA-3'
3'-TTTTTTCCTTTT T TTTTTTCTCTCTCTCT-5'

**1** 5'-*TTTTTTTTCCTTTCACGTT (**E** Site)

**2** 5'-*TTTTTTTTCCTTTCCAGTT (No **E** Site)

**3** 5'-AACGTCTTTTTCTCTCTCTCT (**E** Site)

**4** 5'-AACTGCTTTTTCTCTCTCTCT (No **E** Site)