

2

AD-A237 360



OFFICE OF NAVAL RESEARCH

Grant or Contract N00014-88-K-0441

DTIC
ELECTRONIC
JUN 26 1991
S
C

R&T Code 4135018

Technical Report #1

"Recognition of All Four Base Pairs of Duplex DNA by Triple Helix Formation. Design of Pyrimidine Specific Bases"

by

L. C. Griffin, L. L. Kiessling and P. B. Dervan

California Institute of Technology
Division of Chemistry and Chemical Engineering
Pasadena, CA

June 1, 1991

Reproduction in whole or in part is permitted for any purpose of the United States Government

This document has been approved for public release and sale; its distribution is unlimited.

91 8 20 002

91-03203



**Recognition of All Four Base Pairs of Duplex DNA by Triple Helix Formation.
Design of Pyrimidine Specific Bases.**

The novel base 4-(3-benzamido)phenylimidazole was designed, synthesized and incorporated within a pyrimidine oligonucleotide and shown to recognize pyrimidine-purine base pairs over purine-pyrimidine base pairs. Such specificity allows binding by triple helix formation at an 18 base pairs site in simian virus 40 DNA containing *all four base pairs* at physiologically relevant conditions.



Accession for
ATC 22221
DTIC TAB
Unannounced
Justification

By
Distribution/

Availability Codes

Dist. Period

A-1

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

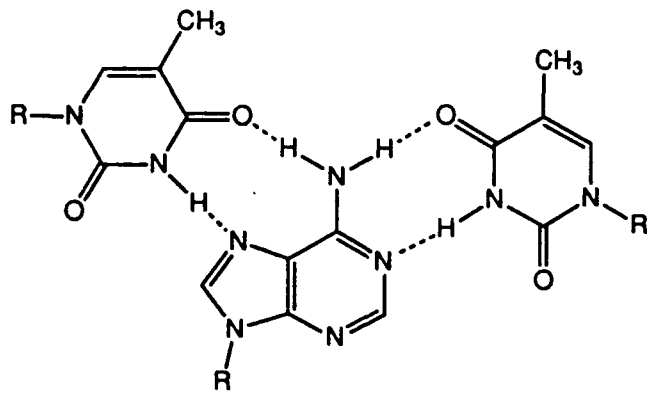
1a. REPORT SECURITY CLASSIFICATION Unclassified		1b. RESTRICTIVE MARKINGS													
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION / AVAILABILITY OF REPORT													
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE		4. PERFORMING ORGANIZATION REPORT NUMBER(S) Report #1.													
6a. NAME OF PERFORMING ORGANIZATION California Institute of Technology		6b. OFFICE SYMBOL (if applicable)													
7a. NAME OF MONITORING ORGANIZATION Office of Naval Research		5. MONITORING ORGANIZATION REPORT NUMBER(S) 4135018													
6c. ADDRESS (City, State, and ZIP Code) Division of Chemistry and Chemical Engineering 164-30 Pasadena, CA 91125		7b. ADDRESS (City, State, and ZIP Code) Department of the Navy Arlington, VA 22217-5000													
8a. NAME OF FUNDING / SPONSORING ORGANIZATION Office of Naval Research		8b. OFFICE SYMBOL (if applicable)													
8c. ADDRESS (City, State, and ZIP Code) 800 No. Quincy St. Arlington, VA 22217-5000		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-88-K-0441													
10. SOURCE OF FUNDING NUMBERS <table border="1" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:25%;">PROGRAM ELEMENT NO.</td> <td style="width:25%;">PROJECT NO.</td> <td style="width:25%;">TASK NO.</td> <td style="width:25%;">WORK UNIT ACCESSION NO.</td> </tr> <tr> <td></td> <td></td> <td></td> <td>4135018</td> </tr> </table>				PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.				4135018				
PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.												
			4135018												
11. TITLE (Include Security Classification) Recognition of All Four Base Pairs of Duplex DNA by Triple Helix Formation. Design of Pyrimidine Specific Bases.															
12. PERSONAL AUTHOR(S) L. C. Griffin, L. L. Kiessling and P. B. Dervan															
13a. TYPE OF REPORT Technical		13b. TIME COVERED FROM 05-31-90 TO 06-01-91													
14. DATE OF REPORT (Year, Month, Day) 91 June 1		15. PAGE COUNT													
16. SUPPLEMENTARY NOTATION															
17. COSATI CODES <table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th style="width:33%;">FIELD</th> <th style="width:33%;">GROUP</th> <th style="width:33%;">SUB-GROUP</th> </tr> </thead> <tbody> <tr><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td></tr> </tbody> </table>			FIELD	GROUP	SUB-GROUP										18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP													
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The novel base 4-(3-benzamido)phenylimidazole was designed, synthesized and incorporated within a pyrimidine oligonucleotide and shown to recognize pyrimidine-purine base pairs over purine-pyrimidine base pairs. Such specificity allows binding by triple helix formation at an 18 base pairs site in simian virus 40 DNA containing all four base pairs at physiologically relevant conditions.															
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> OTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION													
22a. NAME OF RESPONSIBLE INDIVIDUAL Dr. Harold Guard		22b. TELEPHONE (Include Area Code) 202-696-4409													
22c. OFFICE SYMBOL ONR															

Recognition of All Four Base Pairs of Duplex DNA by Triple Helix Formation. Design of Pyrimidine Specific Bases.

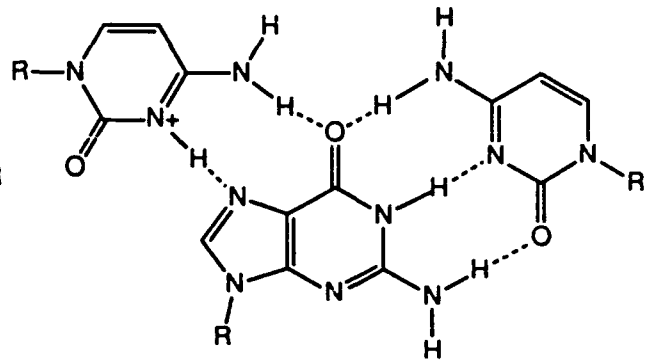
*L. C. Griffin, L. L. Kiessling and P. B. Dervan**

*Arnold and Mabel Beckman Laboratories of Chemical Synthesis
California Institute of Technology
Pasadena, California 91125*

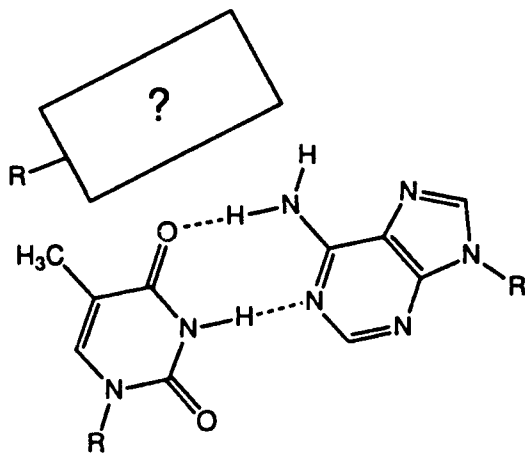
A novel nucleotide equipped with the base, 4-(3-benzamido)phenylimidazole, was designed, synthesized and incorporated within a pyrimidine oligonucleotide. This was shown to recognize CG and TA base pairs over GC and AG base pairs. Such specificity allows binding by triple helix formation at an 18 base pairs site in simian virus 40 DNA containing all four base pairs at physiologically relevant conditions.



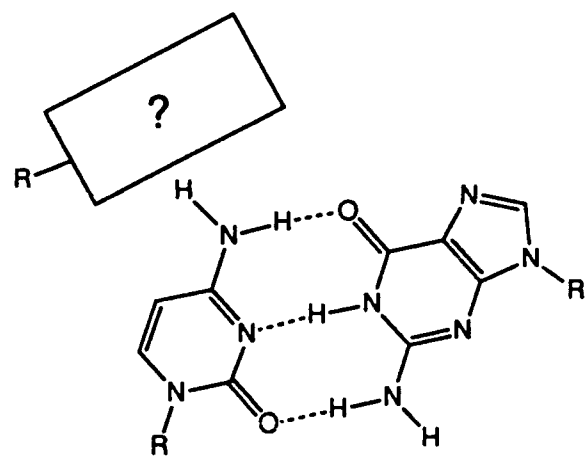
T·AT



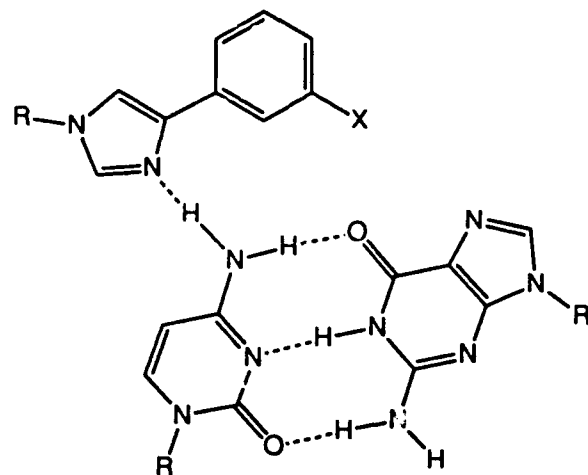
C+GC



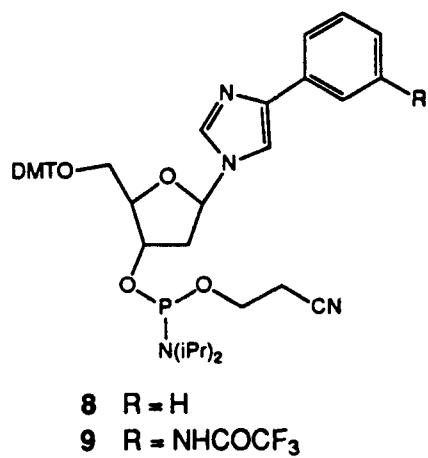
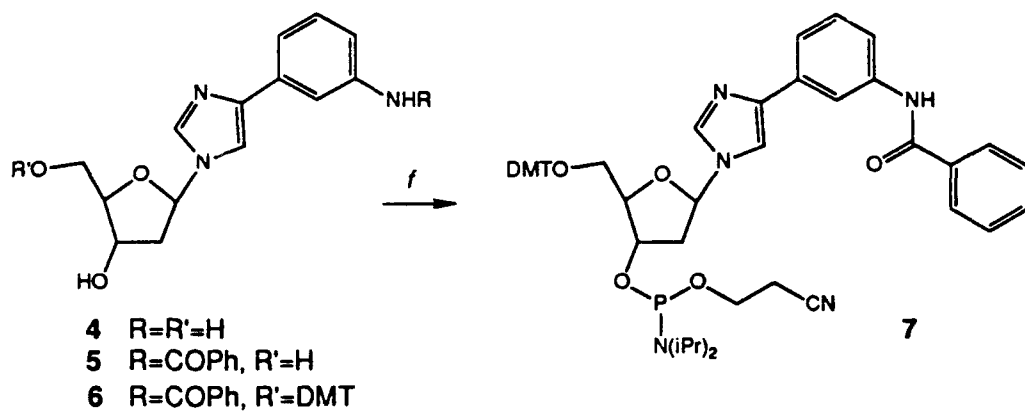
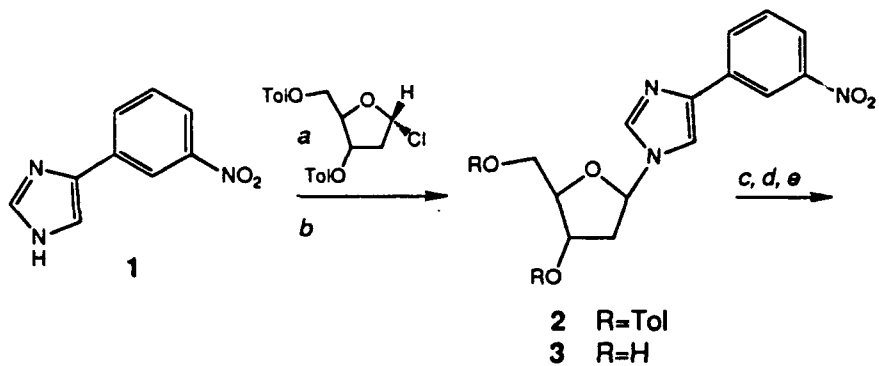
?·TA



?·CG



- D₁** X=H
D₂ X=NH₂
D₃ X=NHCOPh

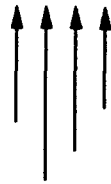


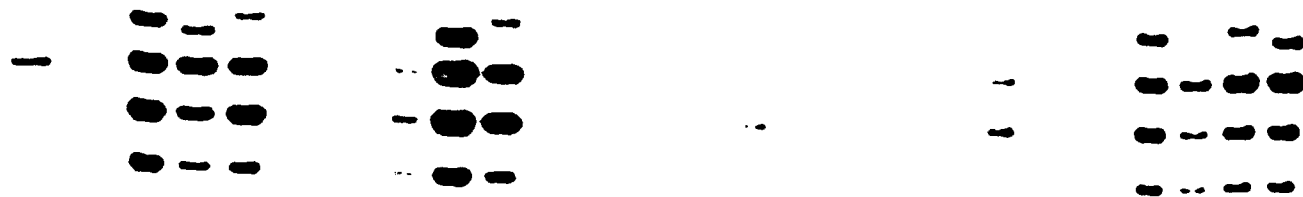
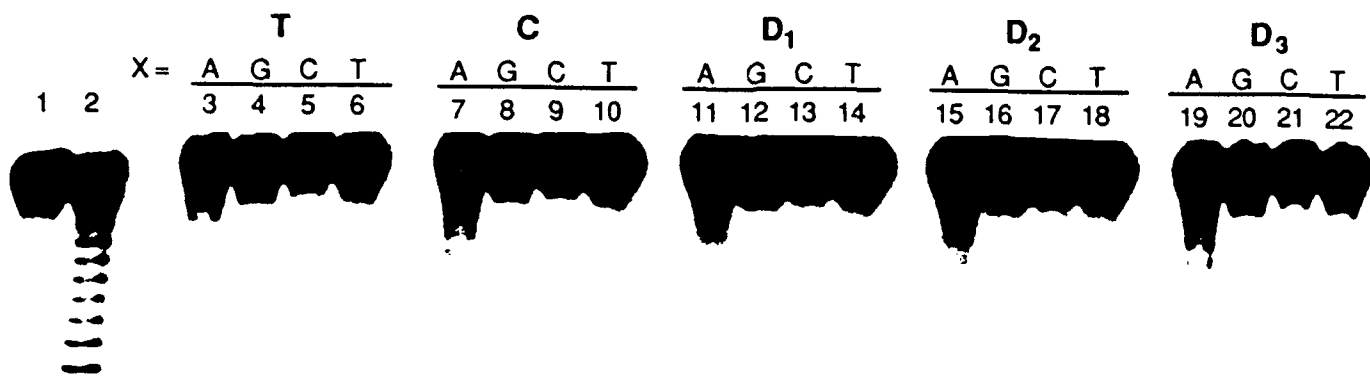
A

5'-TTTT^{*}TTTTTTTTTT-3' 1
5'-TTTT^{*}TTCTTTTTTT-3' 2
5'-TTTT^{*}TTD₁TTTTTTTT-3' 3
5'-TTTT^{*}TTD₂TTTTTTTT-3' 4
5'-TTTT^{*}TTD₃TTTTTTTT-3' 5

5'-CCCCCCCCAA^{*}AAAAAXAAAAAA-3'
3'-GGGGGGGGTT^{*}TTTTTYYTTTTTT-5'

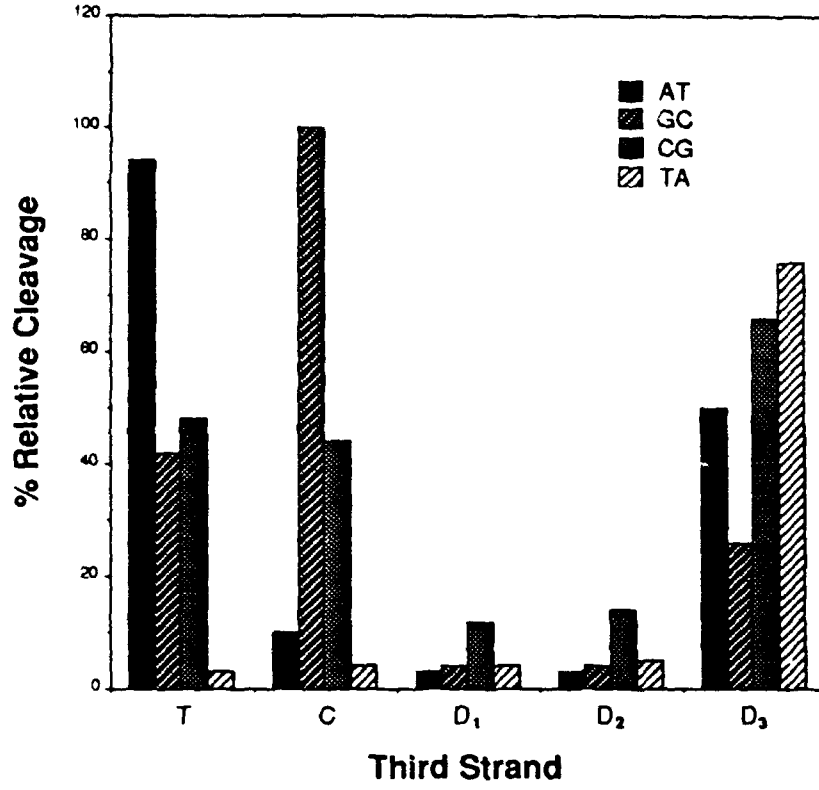
XY = AT, GC, CG, TA



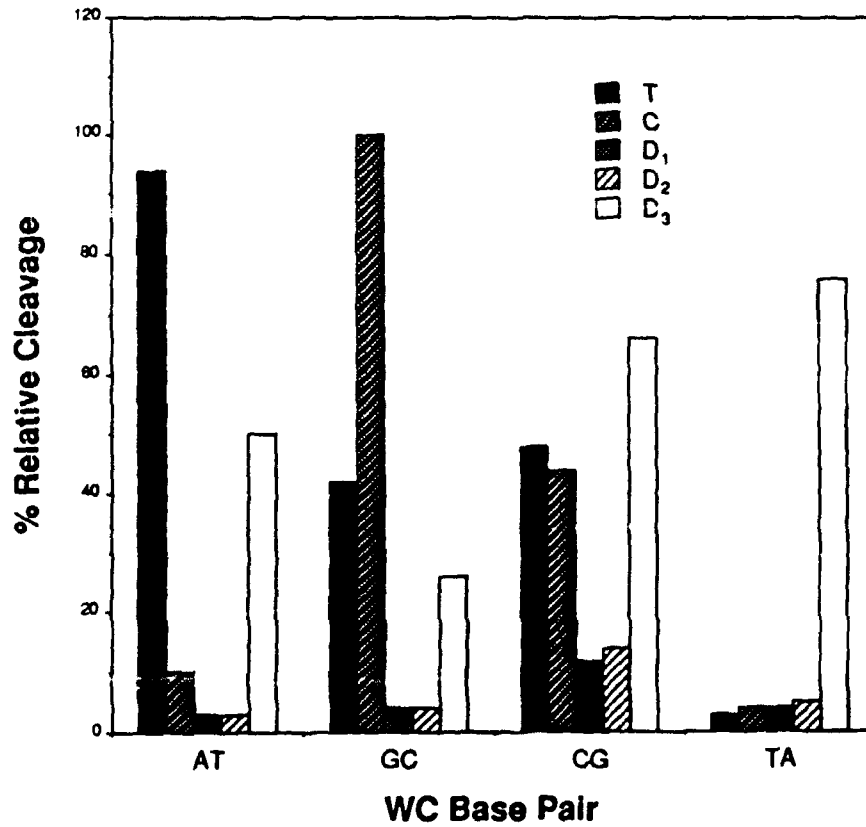


C

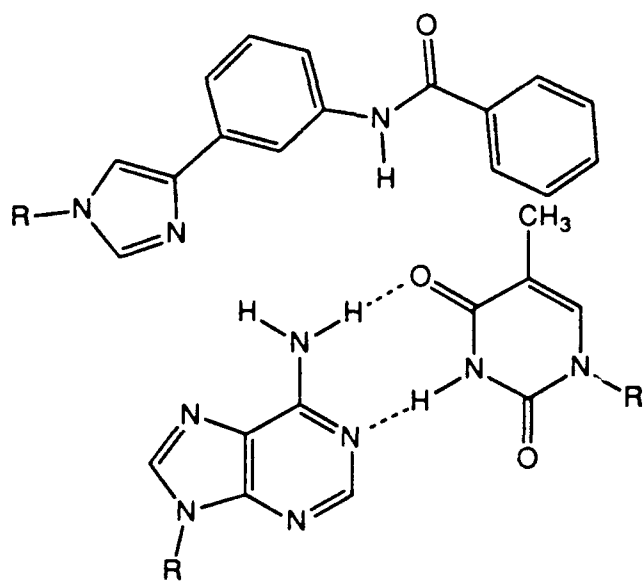
Base Triplets



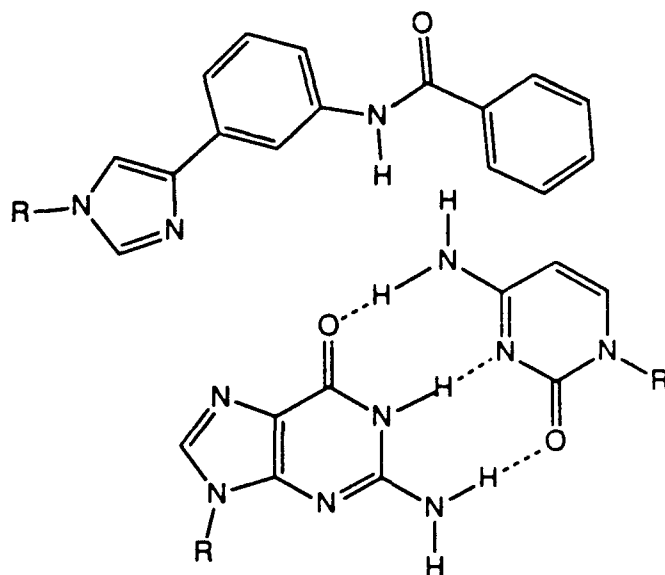
Base Triplets



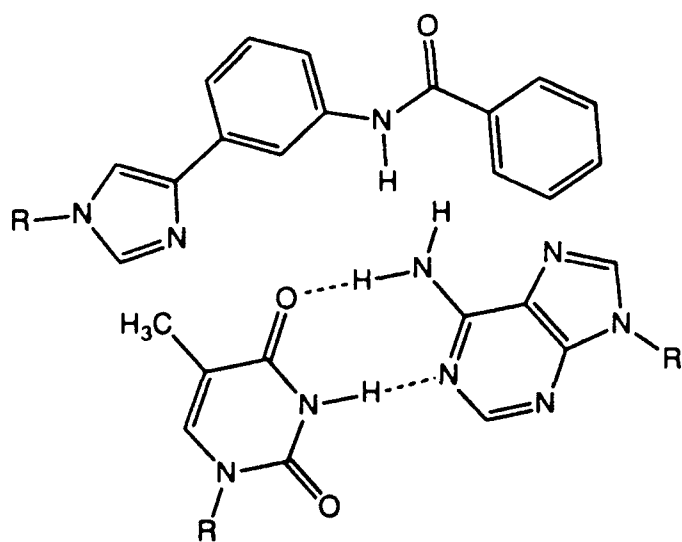
D



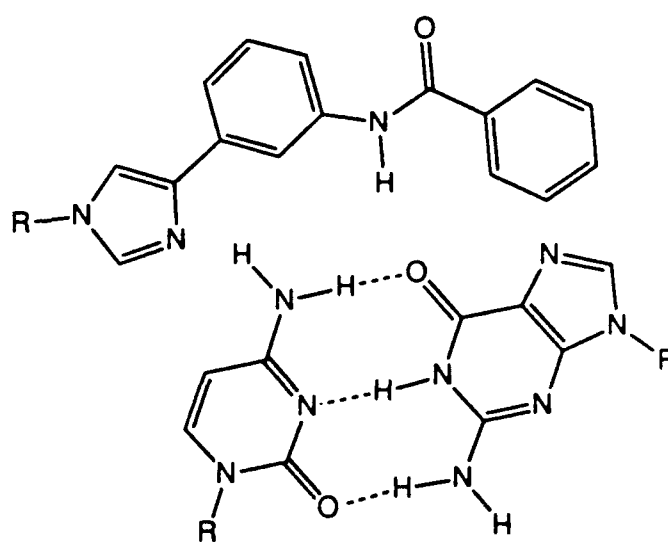
D_3 •AT



D_3 •GC



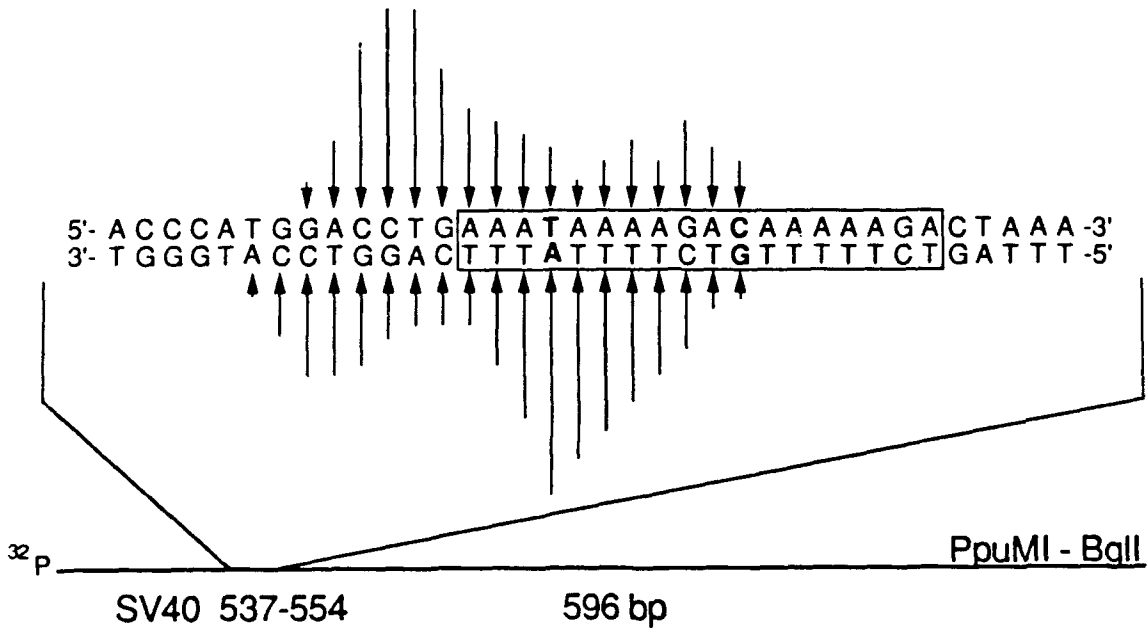
D_3 •TA



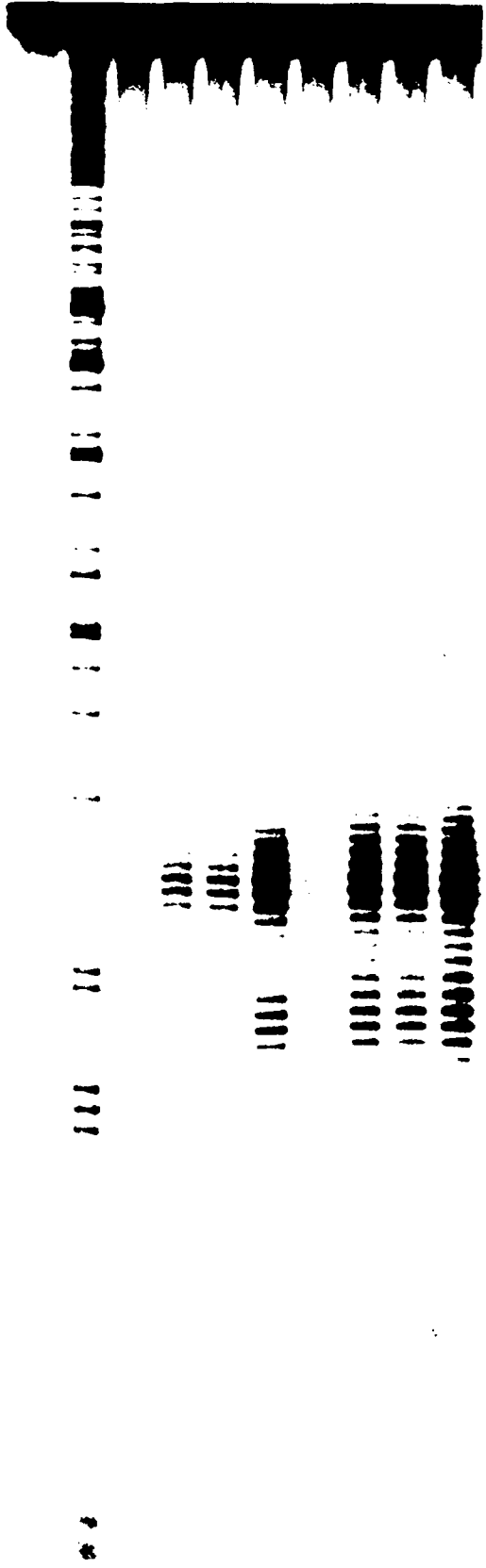
D_3 •CG

A

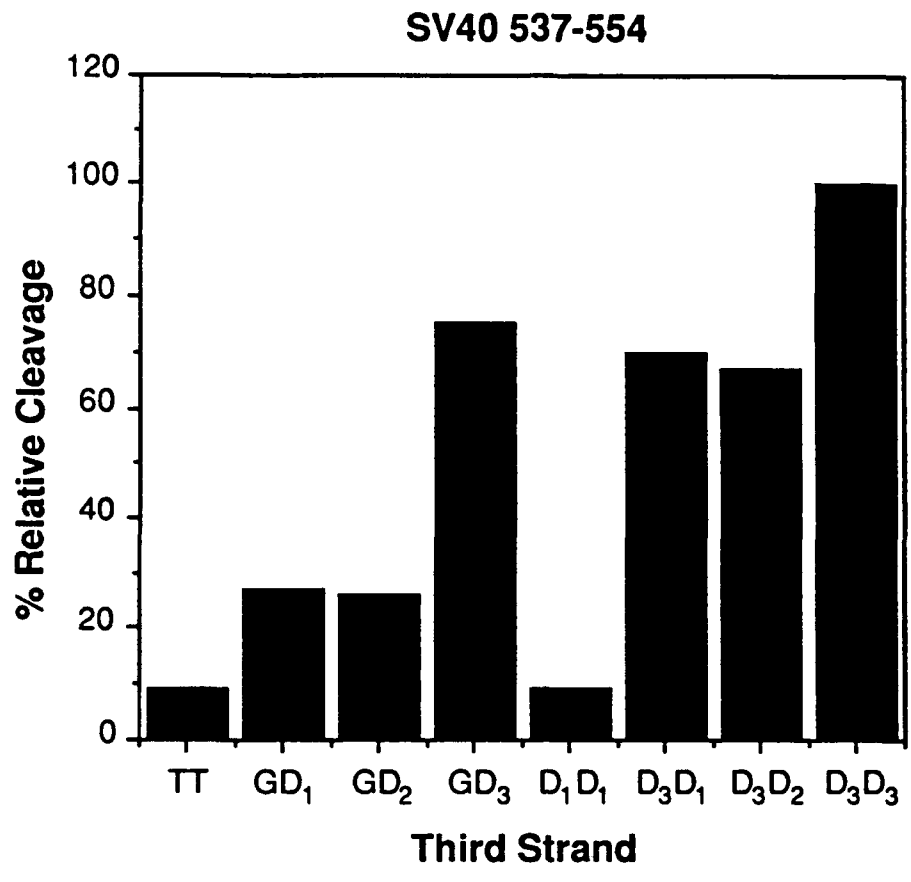
5'-[†]TTTTTTTTCTTTTTTTCT-3' 6
 5'-[†]TTTGTTTTCTD₁TTTTTTCT-3' 7
 5'-[†]TTTGTTTTCTD₂TTTTTTCT-3' 8
 5'-[†]TTTGTTTTCTD₃TTTTTTCT-3' 9
 5'-[†]TTTD₁TTTTCTD₁TTTTTTCT-3' 10
 5'-[†]TTTD₂TTTTCTD₂TTTTTTCT-3' 11
 5'-[†]TTTD₃TTTTCTD₃TTTTTTCT-3' 12
 5'-[†]TTTD₄TTTTCTD₄TTTTTTCT-3' 13

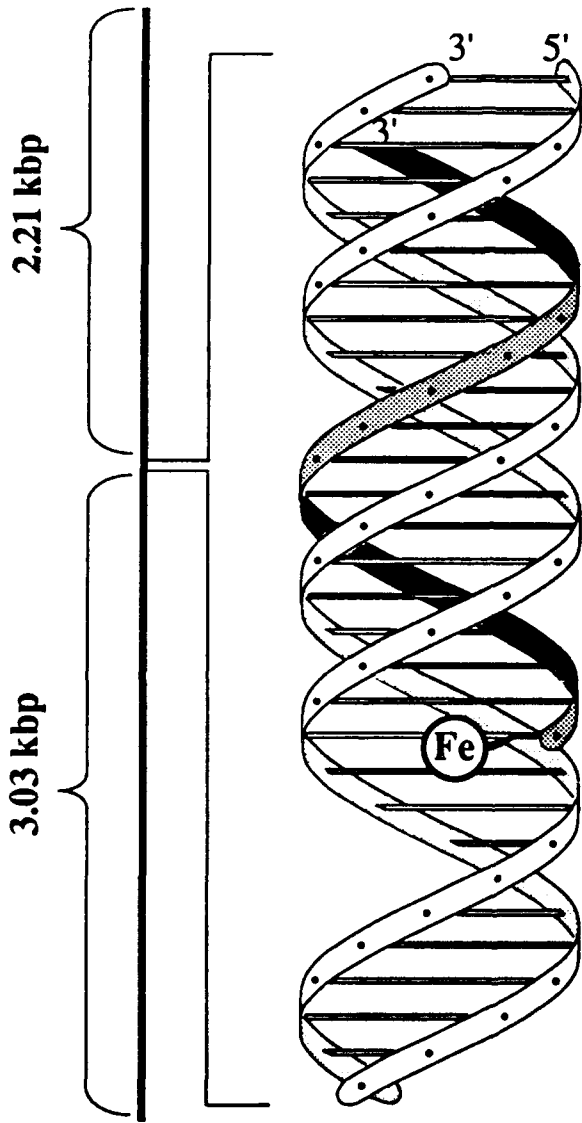


Control	G rxn	TT	GD ₁	GD ₂	GD ₃	D ₁ D ₁	D ₃ D ₁	D ₃ D ₂	D ₃ D ₃
1	2	3	4	5	6	7	8	9	10

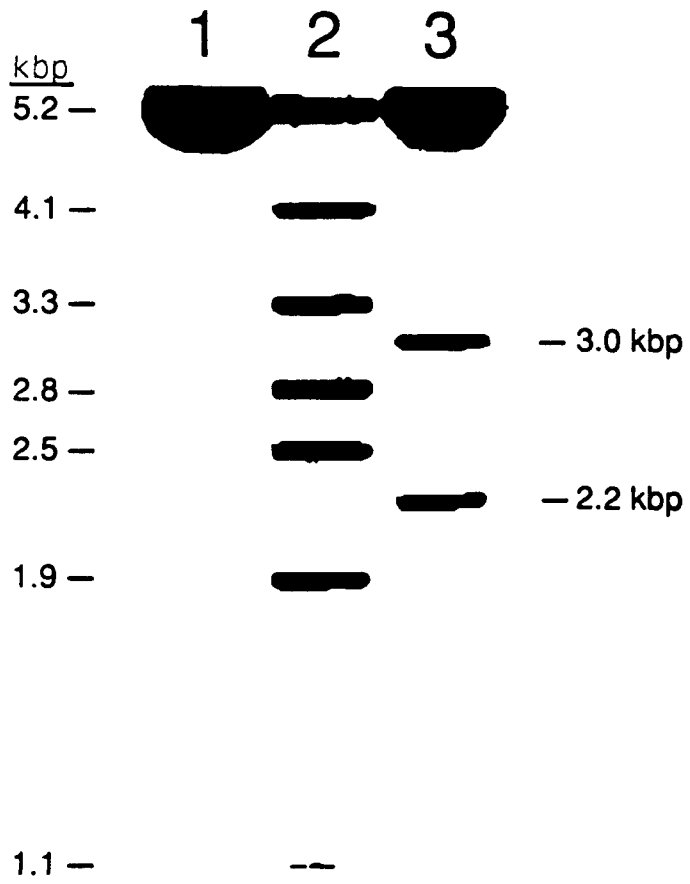


C

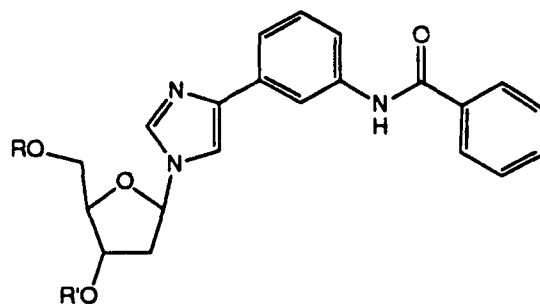




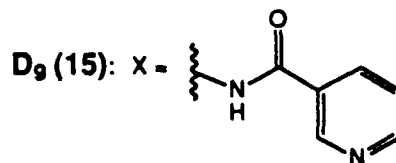
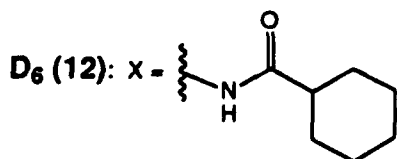
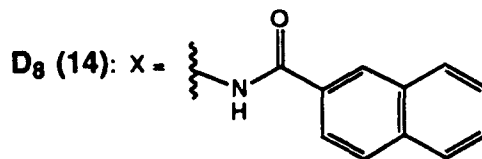
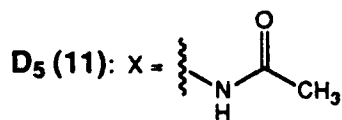
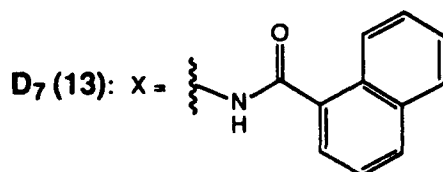
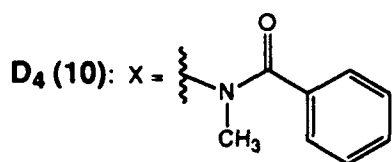
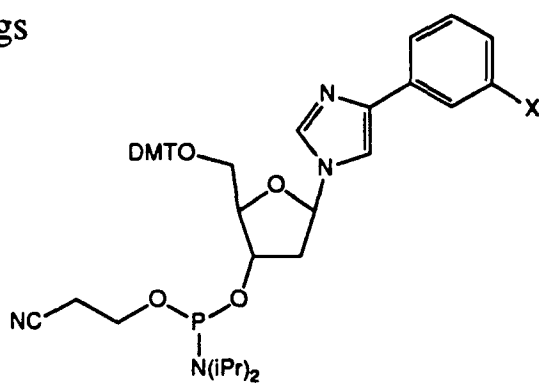
	3'	5'
		T-A
3'	C-G	
T	• A-T	
C	+ G-C	
T	• A-T	
T	• A-T	
T	• A-T	
T	• A-T	
D ₃	• C-G	
T	• A-T	
C	+ G-C	
T	• A-T	
T	• A-T	
T	• A-T	
T	• A-T	
D ₃	• T-A	
T	• A-T	
T	• A-T	
*T	• A-T	
	G-C	
	T-A	
	C-G	
	C-G	
	A-T	
	G-C	
	G-C	
	T-A	
	A-T	
	C-G	



Design Lead: D₃

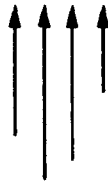


Base Analogs

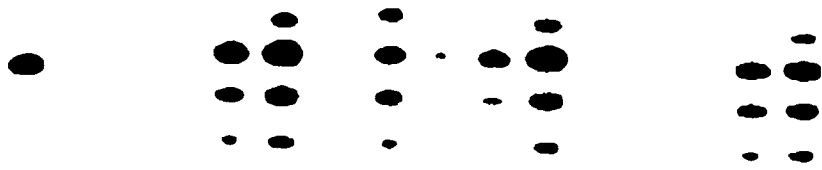
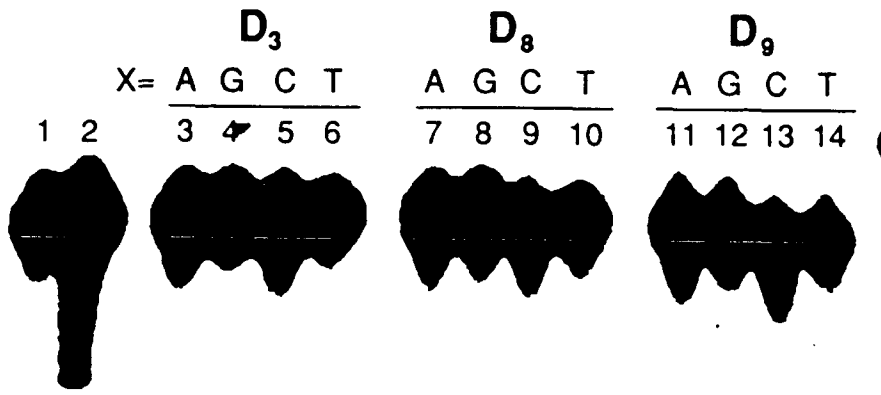


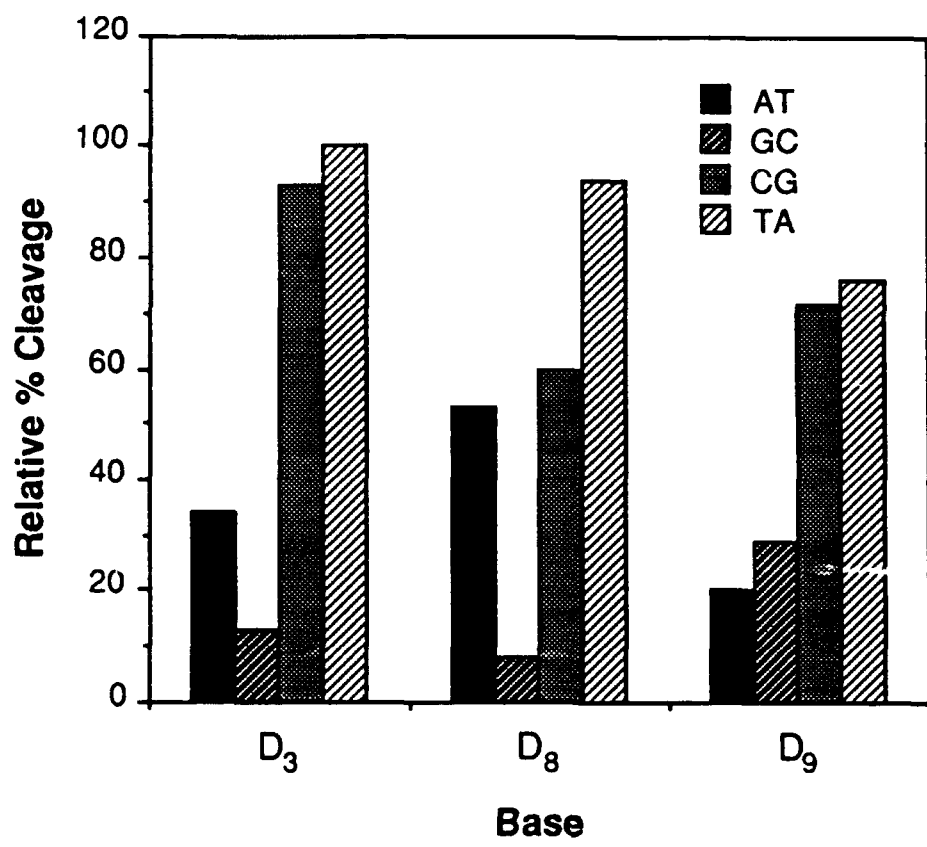
5'- TTTT [*] TTT	D ₃	TTTTTTT	-3'	1
5'- TTTT [*] TTT	D ₄	TTTTTTT	-3'	2
5'- TTTT [*] TTT	D ₅	TTTTTTT	-3'	3
5'- TTTT [*] TTT	D ₆	TTTTTTT	-3'	4
5'- TTTT [*] TTT	D ₇	TTTTTTT	-3'	5
5'- TTTT [*] TTT	D ₈	TTTTTTT	-3'	6
5'- TTTT [*] TTT	D ₉	TTTTTTT	-3'	7

5'- CCCCCCCCCC AAAAAAXAAAAAA TTTTTT -3'
 3'- GGGGGGGGGG TTTTTYYTTTTTT AAAAA -5'

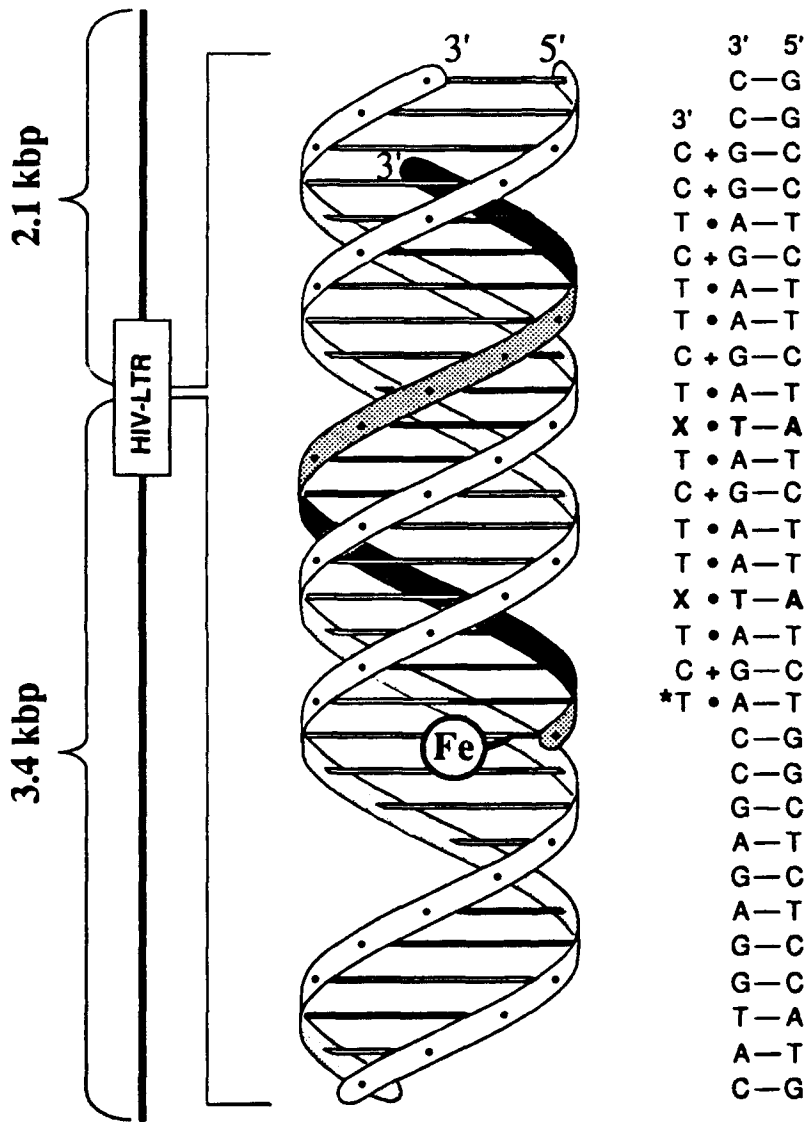


XY = AT, GC, CG, TA





5'-TCTGTTCTGTCTTCTCC-3' 8
 5'-TCTD₃TTCTD₃TCTTCTCC-3' 9
 5'-TCTD₈TTCTD₈TCTTCTCC-3' 10
 5'-TCTD₉TTCTD₉TCTTCTCC-3' 11



		4 °C				23 °C				37 °C			
		G	D ₃	D ₉	D ₈	G	D ₃	D ₉	D ₈	G	D ₃	D ₉	D ₈
1	2	3	4	5	6	7	8	9	10	11	12	13	14

kbp

3.7-

-3.4 kbp

2.3-

1.9-

-2.1 kbp

1.4-

1.3-