Figure 1

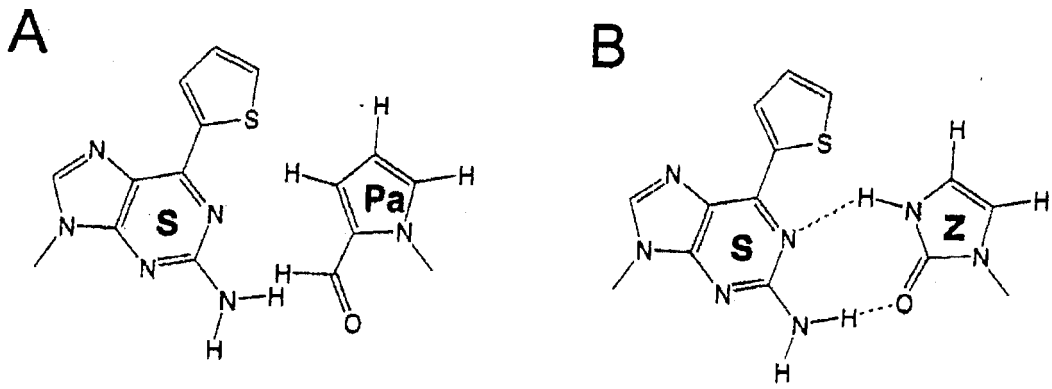


Figure 2

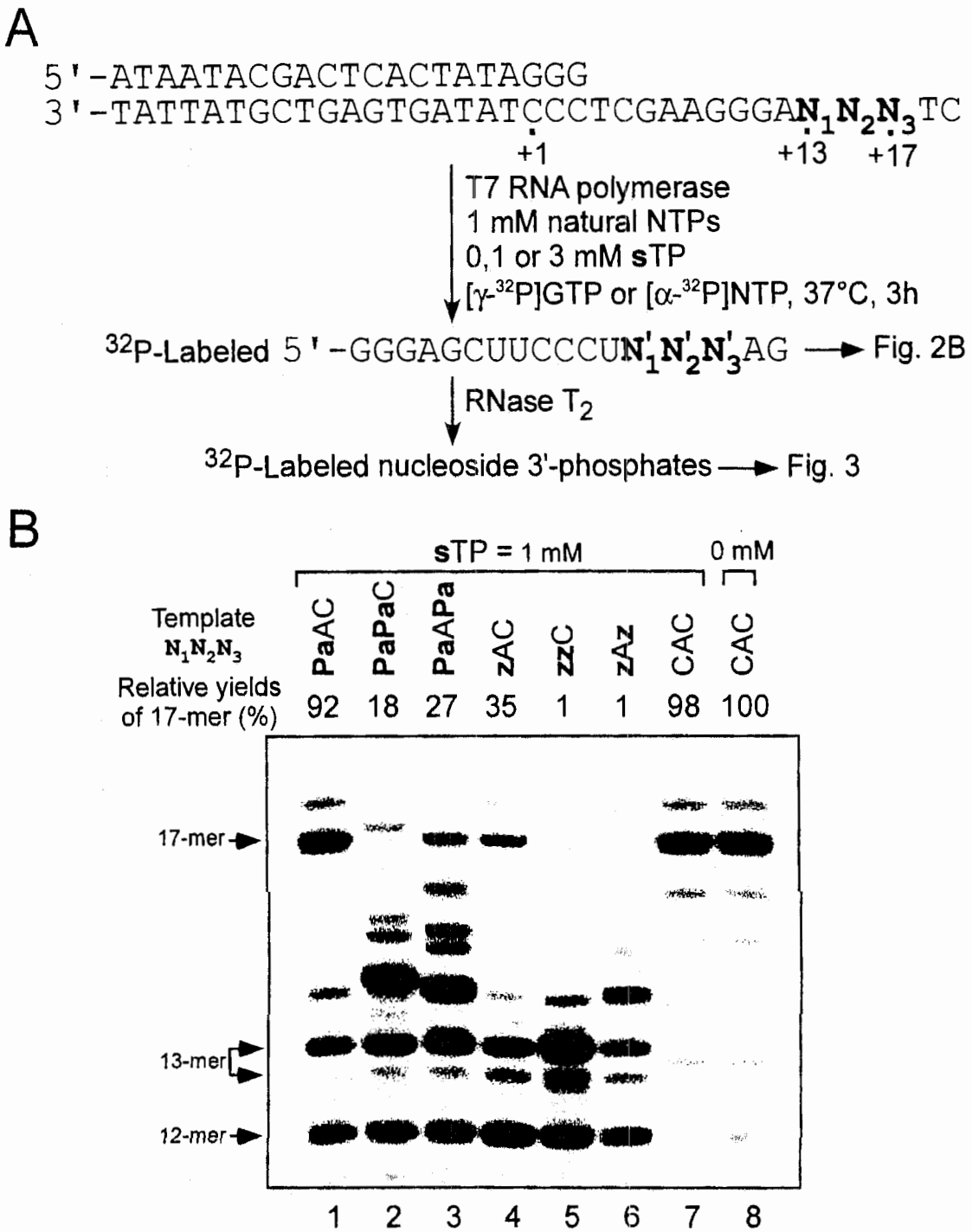

A
5'-ATAATACGACTCACTATAGGG
3'-TATTATGCTGAGTGATATCCCTCGAAGGGAN1N2N3TC
+1
+13
+17
T7 RNA polymerase
1 mM natural NTPs
0,1 or 3 mM sTP
[γ-32P]GTP or [α-32P]NTP, 37°C, 3h
32P-Labeled 5'-GGGAGCUUCCCUN'1N'2N'3AG
Fig. 2B
RNase T2
32P-Labeled nucleoside 3'-phosphates
Fig. 3
B
sTP = 1 mM
0 mM
Template N1N2N3
PaAC
PaPaC
PaAPa
zAC
zzC
zAz
CAC
CAC
Relative yields of 17-mer (%)
92
18
27
35
1
1
98
100
17-mer
13-mer
12-mer
1
2
3
4
5
6
7
8

Figure 3

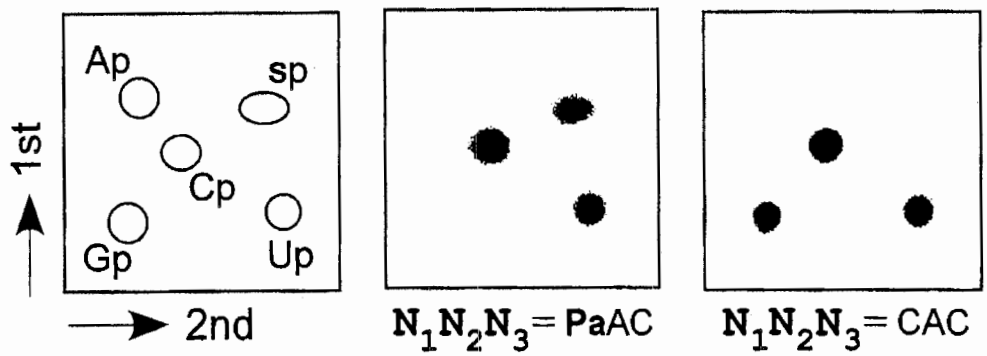


Figure 4

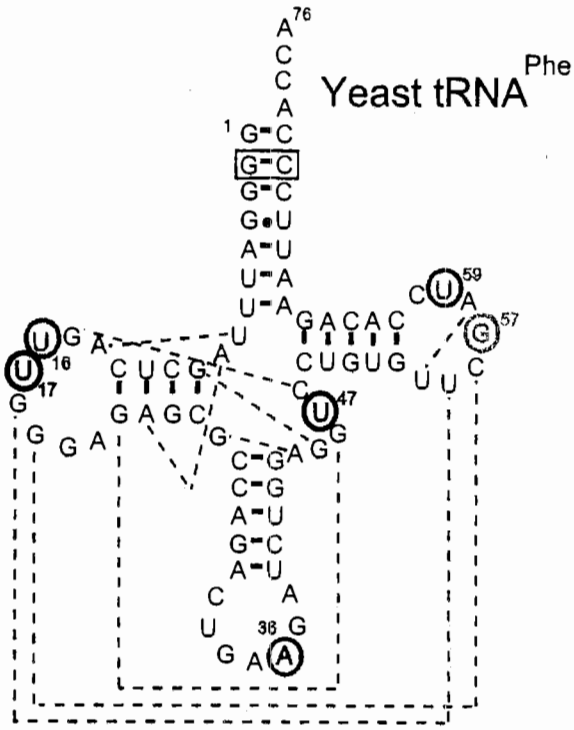


Figure 5

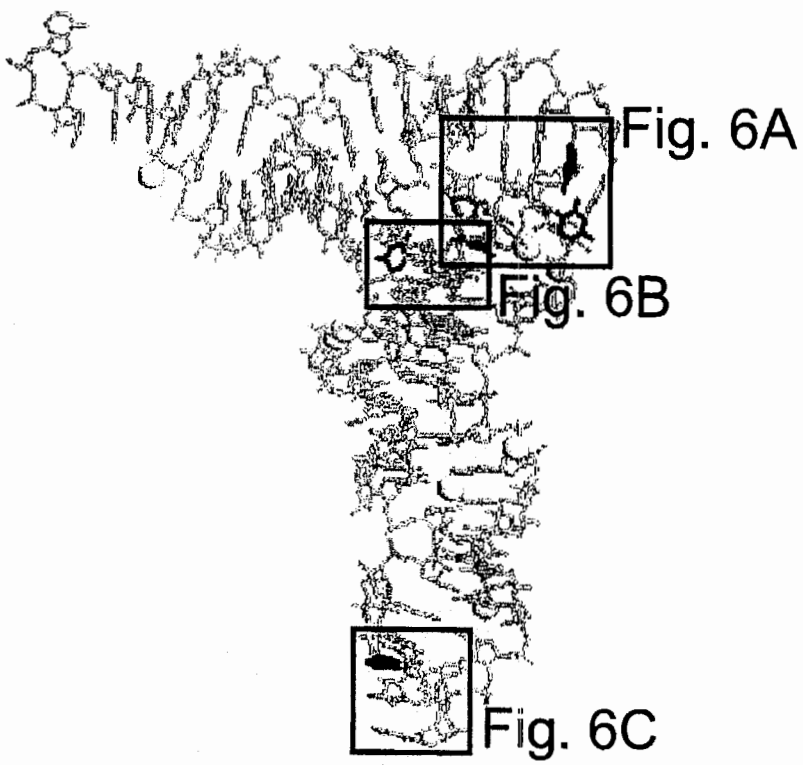


Figure 6

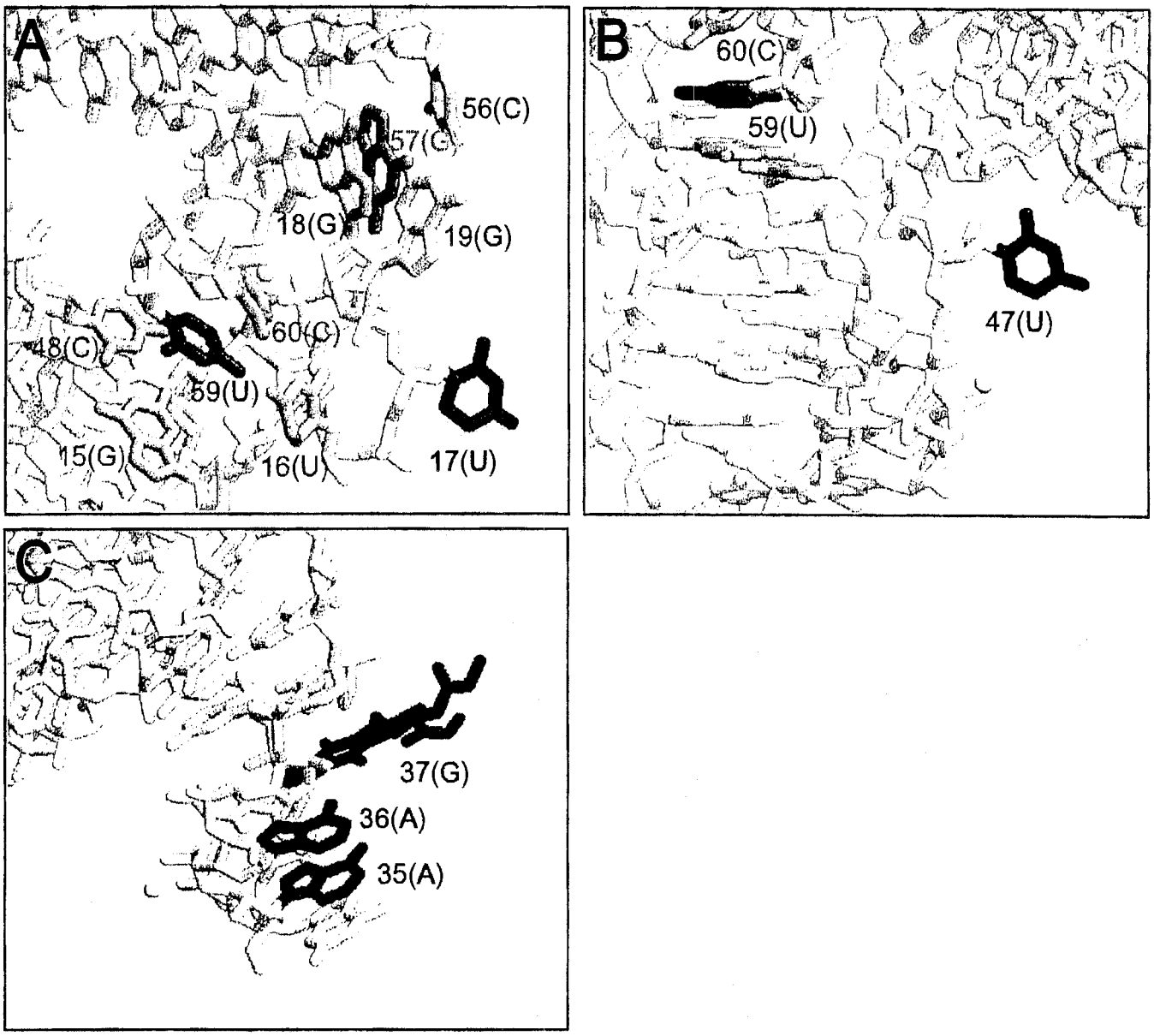

A
56(C)
57(C)
18(G)
19(G)
48(C)
60(C)
59(U)
15(G)
16(U)
17(U)
B
60(C)
59(U)
47(U)
C
37(G)
36(A)
35(A)

Figure 7

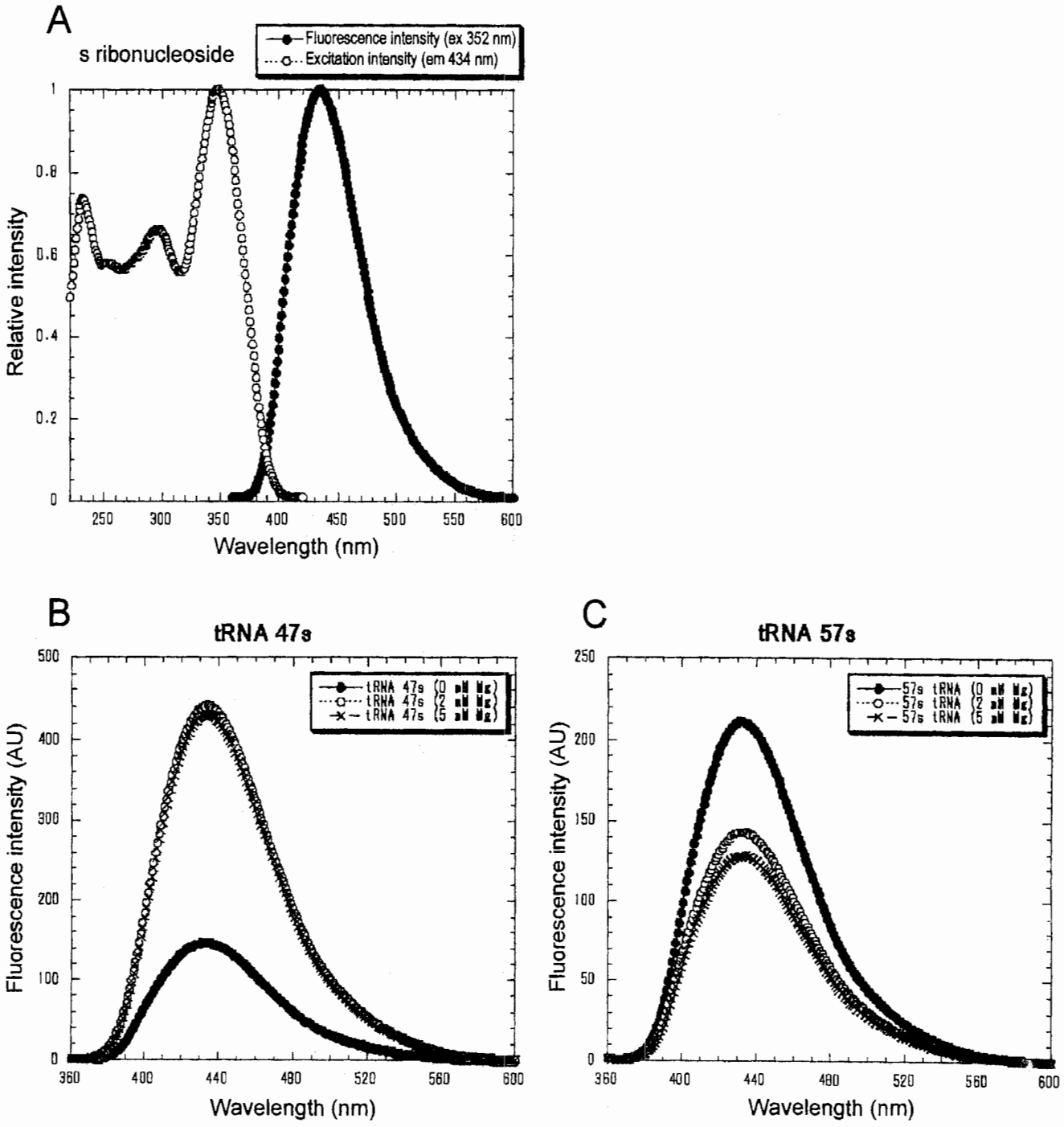

A
s ribonucleoside
Fluorescence intensity (ex 352 nm)
Excitation intensity (em 434 nm)
Relative intensity
Wavelength (nm)
B
tRNA 47s
tRNA 47s (0 mM Mg)
tRNA 47s (2 mM Mg)
tRNA 47s (5 mM Mg)
Fluorescence intensity (AU)
Wavelength (nm)
C
tRNA 57s
57s tRNA (0 mM Mg)
57s tRNA (2 mM Mg)
57s tRNA (5 mM Mg)
Fluorescence intensity (AU)
Wavelength (nm)

Figure 8

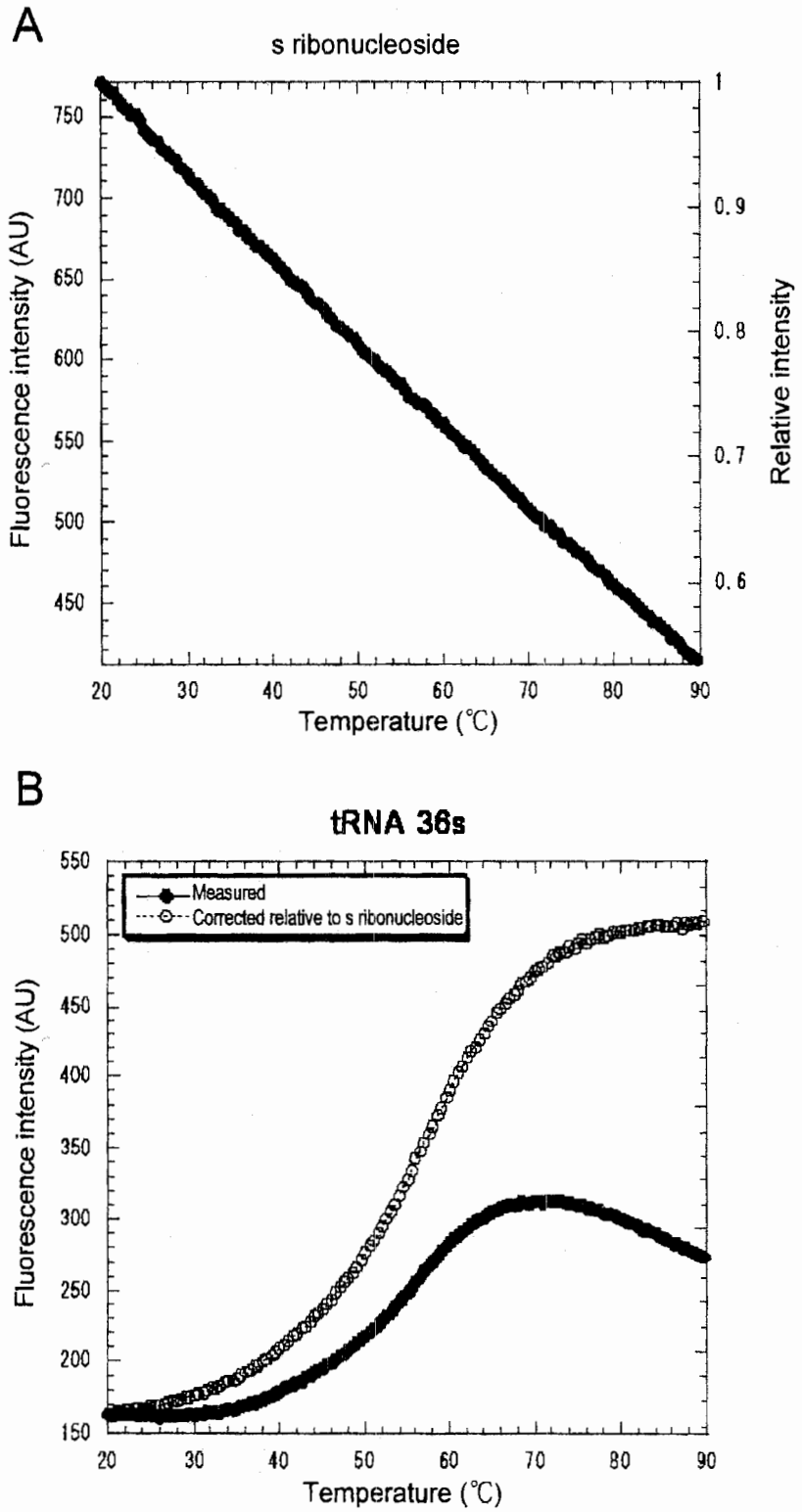

A
s ribonucleoside
Fluorescence intensity (AU)
Relative intensity
Temperature (℃)
B
tRNA 36s
Measured
Corrected relative to s ribonucleoside
Fluorescence intensity (AU)
Temperature (℃)

Figure 9

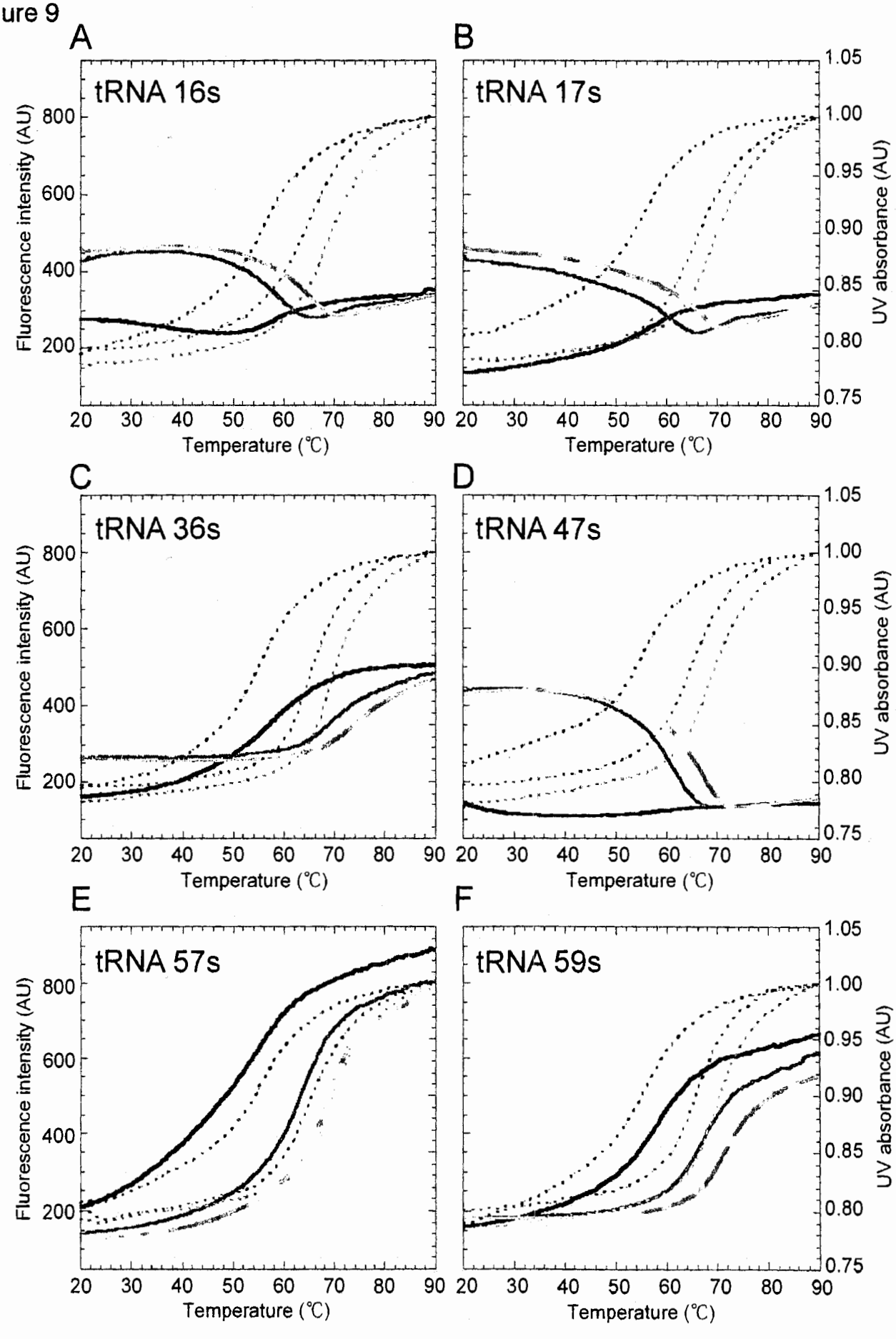

A
tRNA 16s
B
tRNA 17s
C
tRNA 36s
D
tRNA 47s
E
tRNA 57s
F
tRNA 59s
Fluorescence intensity (AU)
UV absorbance (AU)
Temperature (℃)
Fluorescence intensity, 0 mM Mg2+
Fluorescence intensity, 2 mM Mg2+
UV absorbance, 0 mM Mg2+
UV absorbance, 2 mM Mg2+

Figure 10

a

Template DNA

```
NonTemp:Bhp2-cont 5'-AGAATTTAATACGACTCACTATAGGGAATCCCGAGTAGTGCTACTAATAAAATGCACTAGTG
Temp:Bhp2-1       3'-TCTTAAATTATGCTGAGTGATATCCCTTAGGGCTCyTCACGATGATTATTTTACGTGAPCAC
                                  +1          +13                        +36 +39
NonTemp:Bhp2-cont 5'-AGAATTTAATACGACTCACTATAGGGAATCCCGAGTAGTGCTACTAATAAAATGCACTAGTG
Temp:Bhp2-2       3'-TCTTAAATTATGCTGAGTGATATCCCTTAGGGCTCATCyCGATGATTATTTTACGPGATCAC
                                  +1          +16                  +33   +39
NonTemp:Bhp2-cont 5'-AGAATTTAATACGACTCACTATAGGGAATCCCGAGTAGTGCTACTAATAAAATGCACTAGTG
Temp:Bhp2-3       3'-TCTTAAATTATGCTGAGTGATATCCCTTAGGGCTCATCACGyTGATTATTTPACGTGATCAC
                                  +1          +19        +29        +39
```

P in the sequences represents an artificial base Pa.

T7 RNA polymerase
1 mM natural NTPs,
0-1 mM **s**TP, and 0-1 mM yTP
[γ-^{32}P]GTP, 37°C, 3 h

```
5'-32pppGGGAAUCCCGAGNAGNGCNACUAAUAAANUGCNCUNGUG
   +1          +13 +16 +19        +29  +33 +36 +39
```

b

Template	Bhp2-1			Bhp2-2			Bhp2-3		
sTP (mM)	0	0	1	0	0	1	0	0	1
yTP (mM)	0	1	1	0	1	1	0	1	1

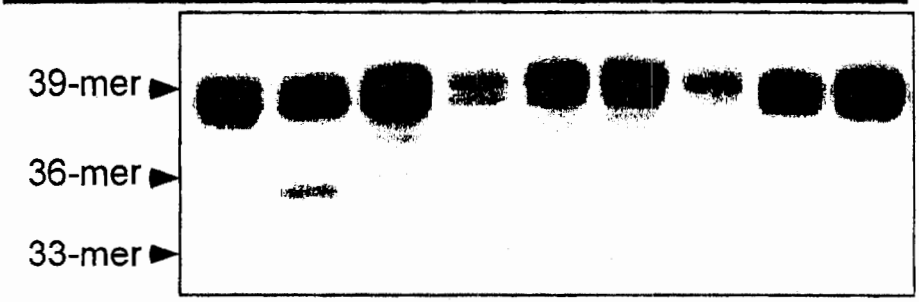


Figure 11

a

NonTemp:Bhp2-cont 5'-AGAATTTAATACGACTCACTATAGGGAATCCCGAGTAGTGCTACTAATAAAATGCACTAGTG
Temp:Bhp2-1 3'-TCTTAAATTATGCTGAGTGATATCCCTTAGGGCTCyTCACGATGATTATTTTACGTGAPCAC
+1 +13 +36 +39

P in the sequences represents an artificial base Pa.

T7 RNA polymerase
1 mM natural NTPs, 10 mM GMP
1 mM sTP, and 0.5 mM yTP
[α-^{32}P]NTP , 37°C, 3 h

^{32}P-labeled 5'-GGGAAUCCCGAGyAGUGCUACUAAUAAAAUGCACUsGUG
+1 +13 +36 +39

RNase T_2

^{32}P-Labeled Ap x 5 + Gp x 2 + Cp x 1 + Up x 3 + sp x 0 + yp x 1 ([α-^{32}P]ATP) (Table, entry 1)
^{32}P-Labeled Ap x 2 + Gp x 1 + Cp x 1 + Up x 3 + sp x 1 + yp x 0 ([α-^{32}P]GTP) (Table, entry 3)
^{32}P-Labeled Ap x 2 + Gp x 2 + Cp x 2 + Up x 1 + sp x 0 + yp x 0 ([α-^{32}P]CTP) (Table, entry 5)
^{32}P-Labeled Ap x 3 + Gp x 2 + Cp x 3 + Up x 0 + sp x 0 + yp x 0 ([α-^{32}P]UTP) (Table, entry 7)

b

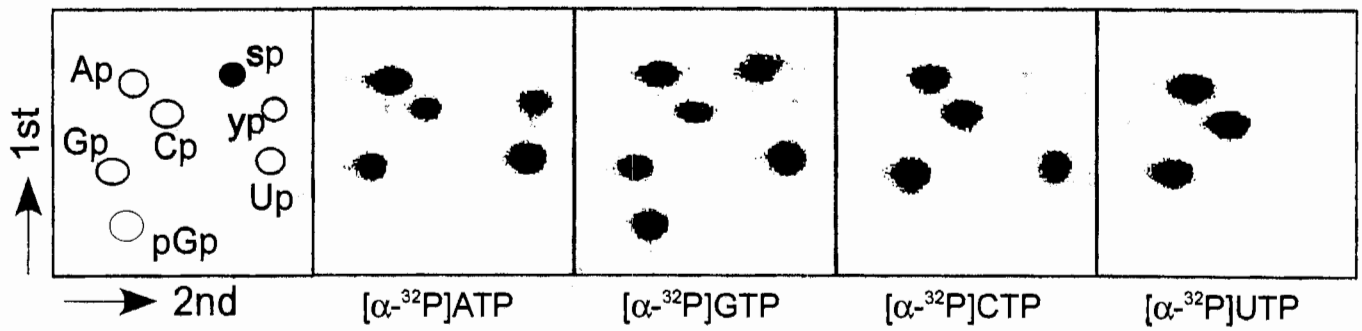


Figure 12

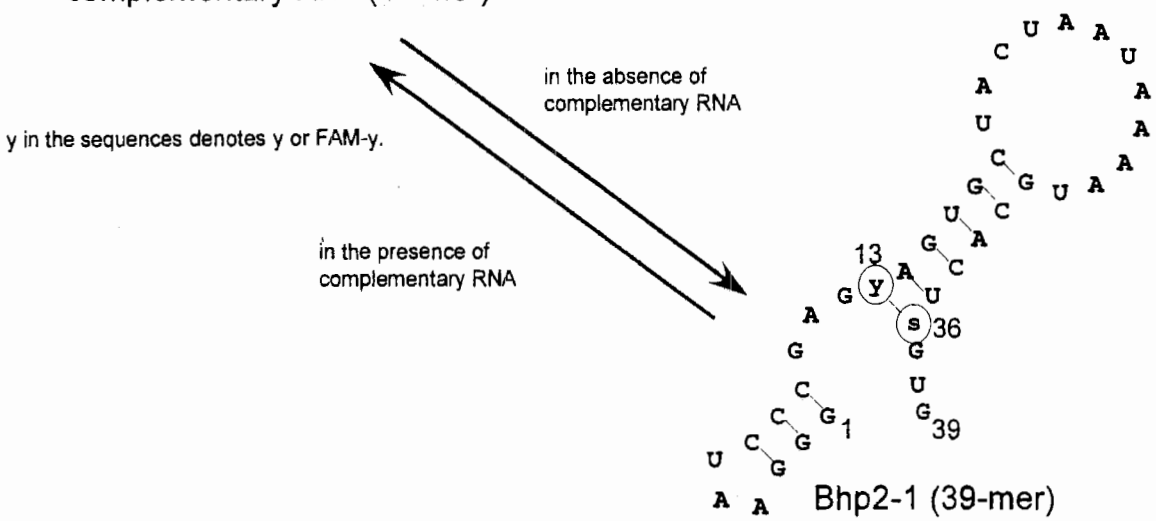
a

Bhp2-1 (39-mer)

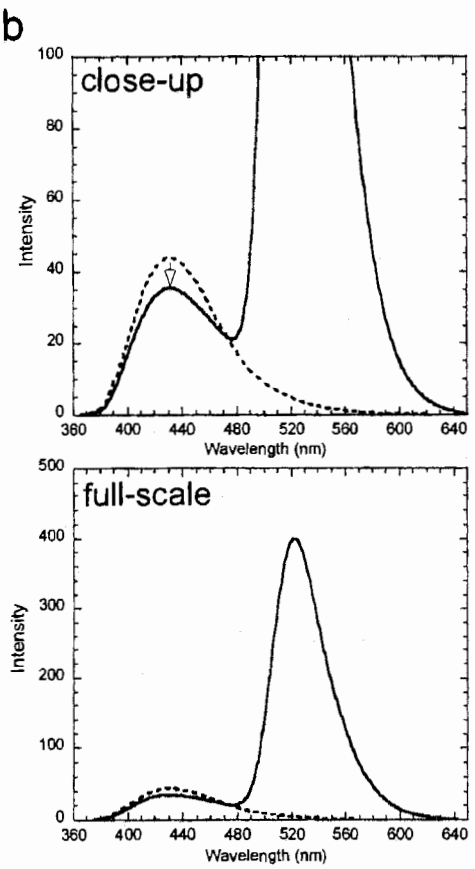
5'-GGGAAUCCCGAGyAGUGCUACUAAUAAAAUGCACUsGUG

3'-CCCUUAGGGCUCAUCACGAUGAUUAUUUUACGUGAUCACGGG

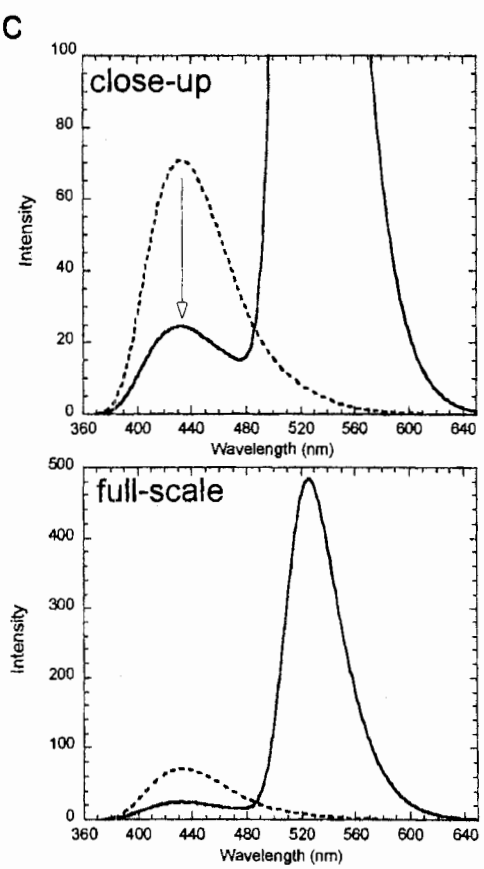
complementary RNA (42-mer)



b



c



Duplex with complementary RNA

Hairpin

---------- Bhp2-1 having y at position 13 and s at position 36

———— Bhp2-1 having FAM-y at position 13 and s at position 36

Figure 13

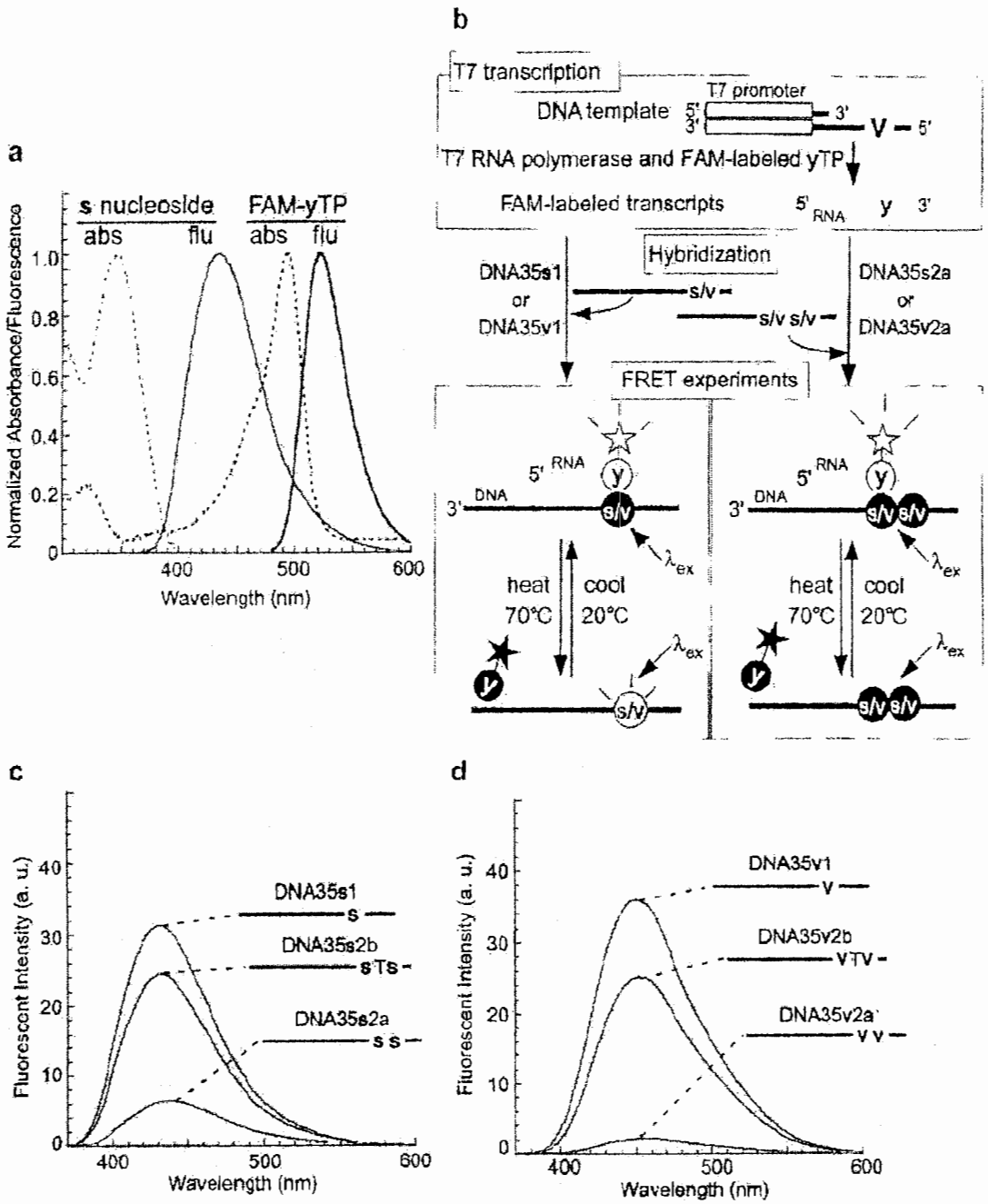

a
s nucleoside
abs flu
FAM-yTP
abs flu
Normalized Absorbance/Fluorescence
Wavelength (nm)
b
T7 transcription
T7 promoter
DNA template
T7 RNA polymerase and FAM-labeled yTP
FAM-labeled transcripts
Hybridization
DNA35s1 or DNA35v1
DNA35s2a or DNA35v2a
FRET experiments
heat 70°C
cool 20°C
c
DNA35s1
DNA35s2b
DNA35s2a
Fluorescent Intensity (a. u.)
Wavelength (nm)
d
DNA35v1
DNA35v2b
DNA35v2a
Fluorescent Intensity (a. u.)
Wavelength (nm)

Figure 14

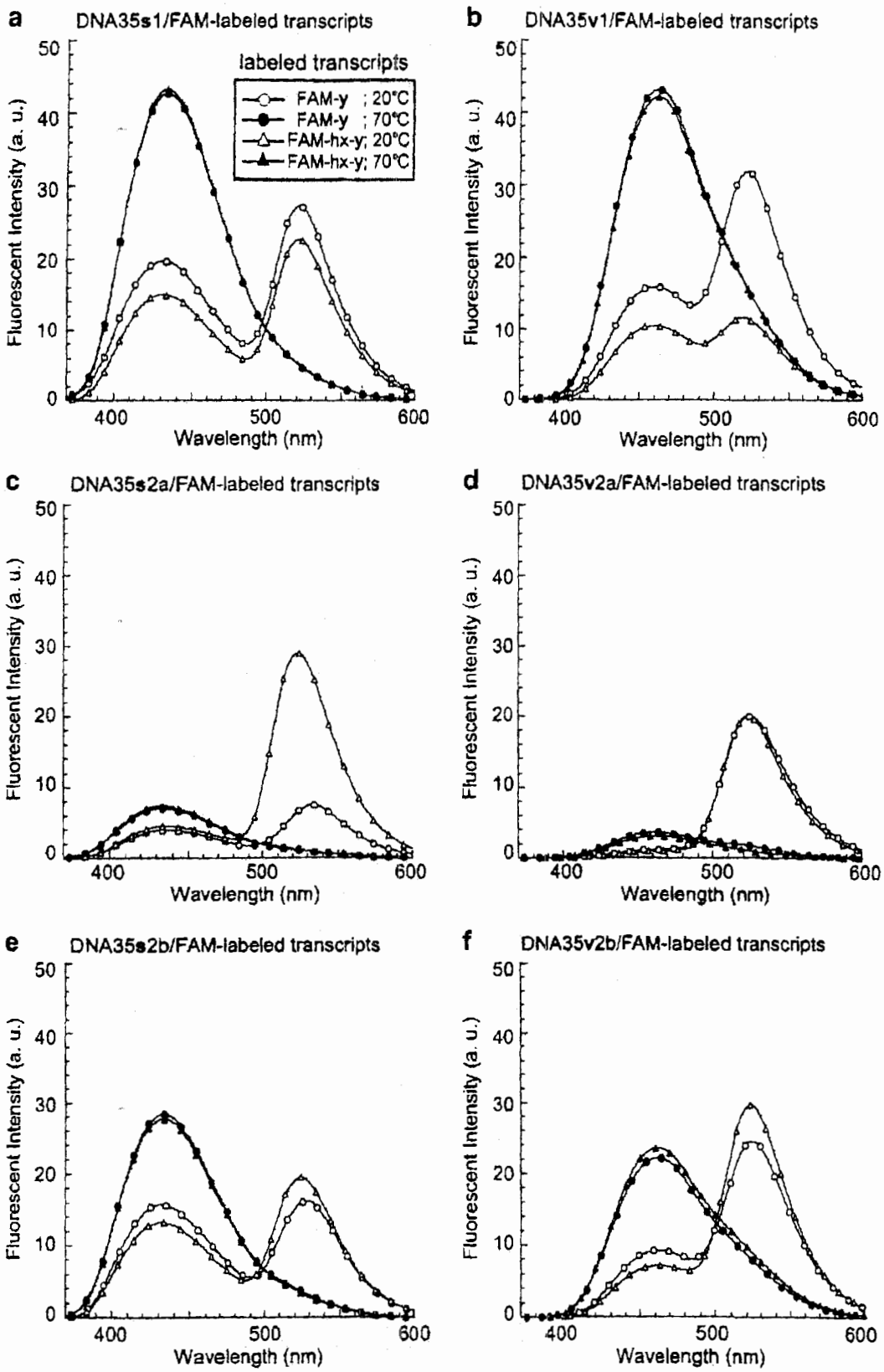
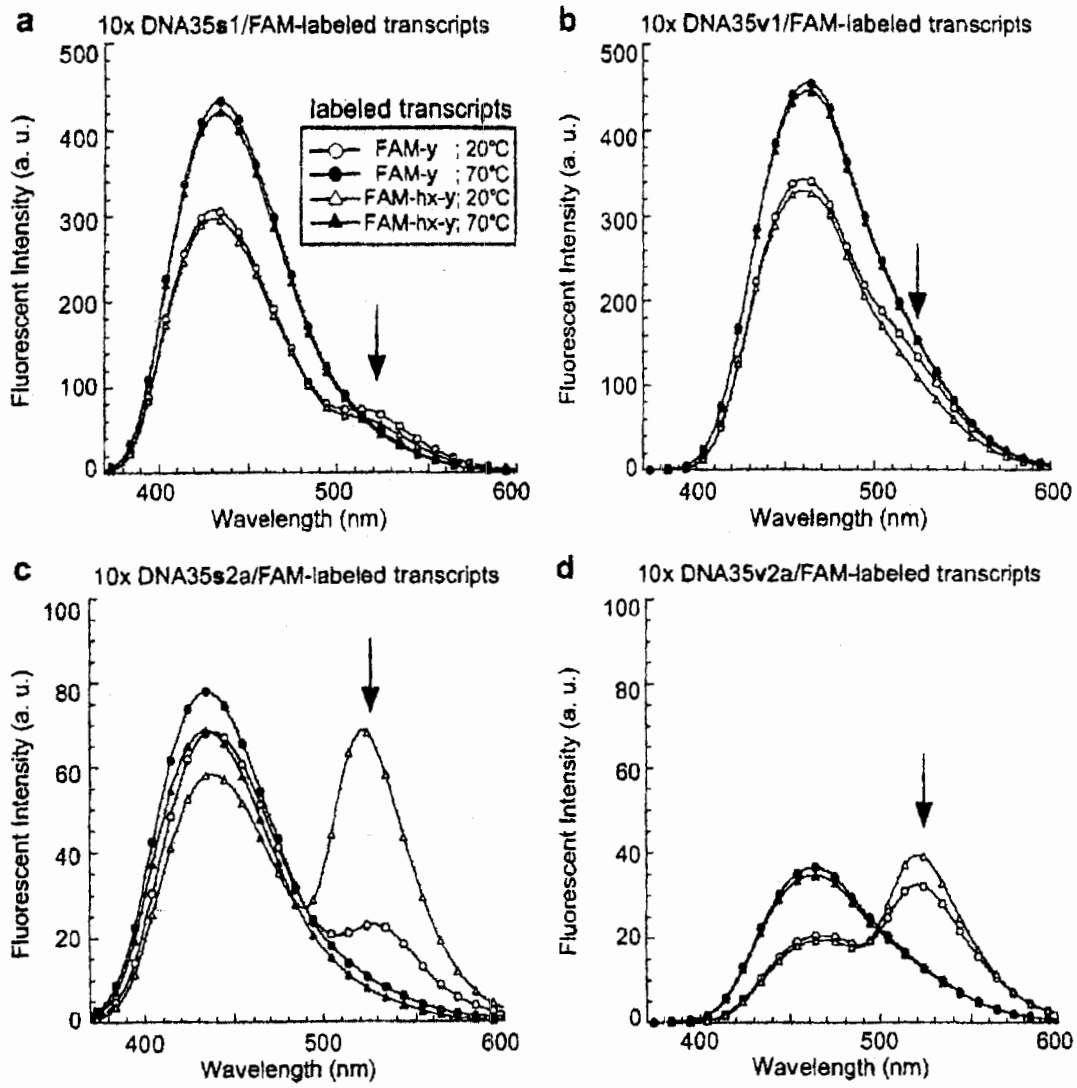

a
DNA35s1/FAM-labeled transcripts
labeled transcripts
FAM-y ; 20°C
FAM-y ; 70°C
FAM-hx-y; 20°C
FAM-hx-y; 70°C
Fluorescent Intensity (a. u.)
Wavelength (nm)
b
DNA35v1/FAM-labeled transcripts
c
DNA35s2a/FAM-labeled transcripts
d
DNA35v2a/FAM-labeled transcripts
e
DNA35s2b/FAM-labeled transcripts
f
DNA35v2b/FAM-labeled transcripts

Figure 15


a
10x DNA35s1/FAM-labeled transcripts
labeled transcripts
FAM-y ; 20°C
FAM-y ; 70°C
FAM-hx-y; 20°C
FAM-hx-y; 70°C
Fluorescent Intensity (a. u.)
Wavelength (nm)
b
10x DNA35v1/FAM-labeled transcripts
Fluorescent Intensity (a. u.)
Wavelength (nm)
c
10x DNA35s2a/FAM-labeled transcripts
Fluorescent Intensity (a. u.)
Wavelength (nm)
d
10x DNA35v2a/FAM-labeled transcripts
Fluorescent Intensity (a. u.)
Wavelength (nm)