

A thymidine triphosphate shape analog  
lacking Watson-Crick pairing ability is  
replicated with high sequence selectivity

*Moran, Ren and Kool*

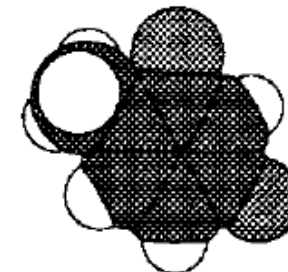
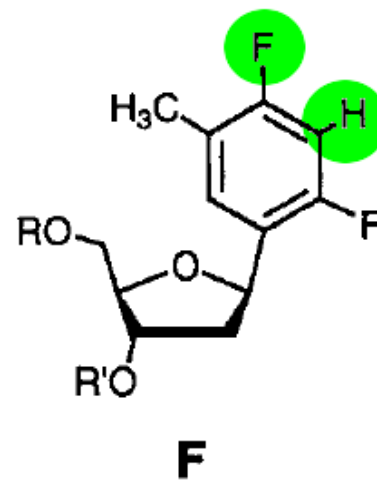
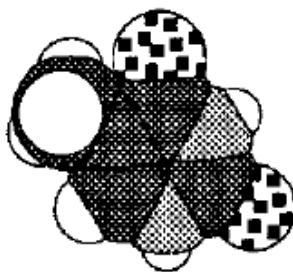
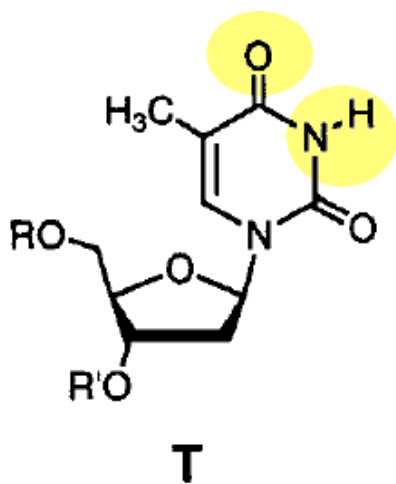
Presentation by *Warren Cheung*

## Main Thrust

Replication uses *shape* recognition

## A thymidine triphosphate shape analog ...

- Compound 1 = F = Difluorotoluene nucleoside
- F is the same shape (space-filling) as T



... lacking Watson-Crick pairing ability ...

- cannot form the hydrogen bonds for base pairing

... is replicated with high sequence selectivity

- tends to pair up with A *during replication*

## Binding “au naturel”

- measure properties of a 12 base long DNA strand
- at one position, they substitute an T-X or F-X base pair
- increase temperature, measure when the strand comes apart
  - melting temperature ( $T_m$ ), change in free energy ( $\Delta G$ )

Table 1. Free energies [ $-\Delta G_{25}^{\circ}$  (kcal)] and melting temperatures [ $T_m$  ( $^{\circ}\text{C}$ )] for duplexes containing a variable T-X or F-X base pair

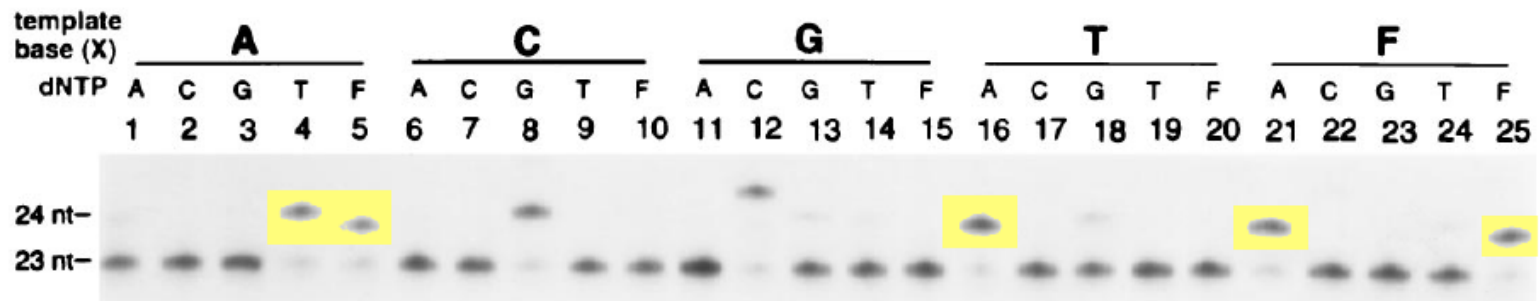
Duplex	$T_m$ ( $^{\circ}\text{C}$ )*	$-\Delta G_{25}^{\circ}$ (kcal)
5'-CTTTTC[TTT]CTT 3'-GAAAAG[AAA]GAA	39.4	12.3
5'-CTTTTC[TTT]CTT 3'-GAAAAG[CA]AGAA	26.4	8.7
5'-CTTTTC[TTT]CTT 3'-GAAAAG[GA]AGAA	30.7	9.3
5'-CTTTTC[TTT]CTT 3'-GAAAAG[TA]AGAA	27.1	8.9
5'-CTTTTC[FTT]CTT 3'-GAAAAG[AAA]GAA	27.4	8.7
5'-CTTTTC[FTT]CTT 3'-GAAAAG[CA]AGAA	22.0	7.7
5'-CTTTTC[FTT]CTT 3'-GAAAAG[GA]AGAA	25.2	8.7
5'-CTTTTC[FTT]CTT 3'-GAAAAG[TA]AGAA	26.5	9.4

\*Error in  $T_m$  values is estimated at  $\pm 0.5^{\circ}\text{C}$ , and in  $\Delta G^{\circ}$  values,  $\pm 10\%$ .

## **F does not pair selectively with A**

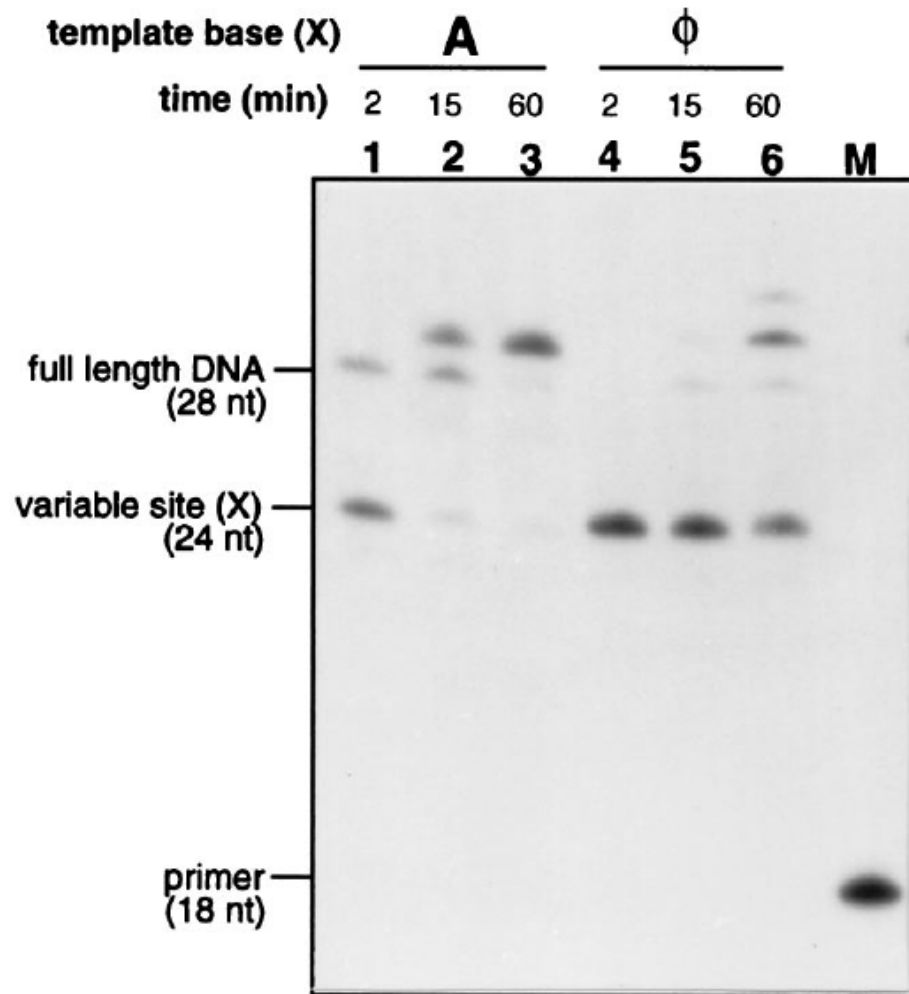
- show that F does not bind selectively with A,G,C,T
- in contrast, T-A is strongest
  - better than F-X pairs, other T-X pairs

## dFTP can be used in DNA polymerisation



- add dFTP in addition to the usual dNTPs
- get  $F \rightarrow A$ ,  $A \rightarrow F$ ,  $F \rightarrow F$  (inserted base  $\rightarrow$  template base)



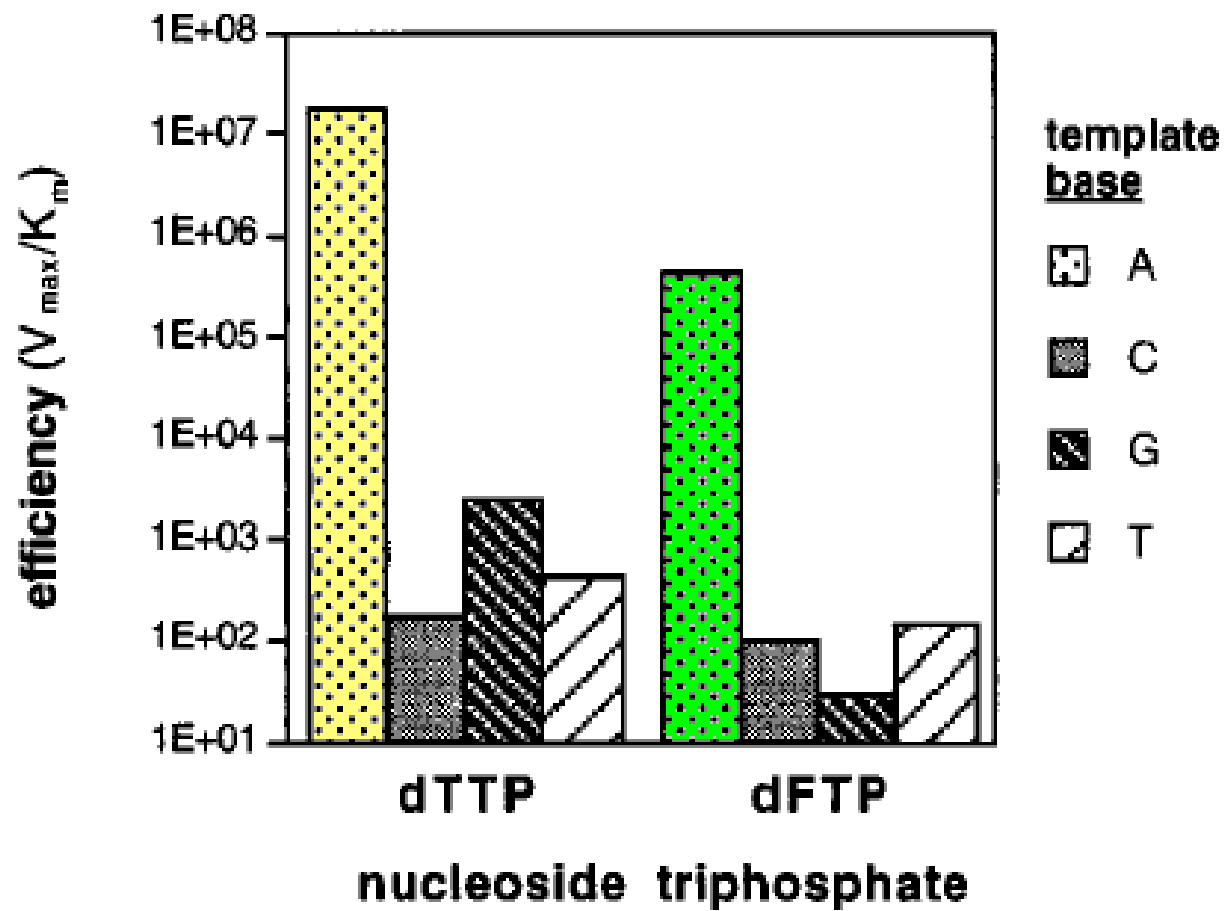


- **Lane A:** insertion across from A (use dFTP instead of dTTP)
- **Lane  $\phi$ :** insertion across abasic site (use normal nucleotides)

## Inserting dFTP causes a brief pause

- dFTP inserted against template A — 2 minute pause
- get a long pause if dFTP is not available
- inserting base against abasic template — 60 minute pause

## Efficiency of Pairing



## **F tends to be paired with a template A by DNA polymerase**

- also tends to be paired with template F (slightly less often)
- all other template bases tend to be much less frequent
- only 40 times less efficient than T pairing with A
- note that C,G,T are  $7 \times 10^3$  to  $9 \times 10^4$  less efficient
- A-rule does not apply (A-rule is when A tends to pair with a basic template)

## **Their Conclusions**

- In the absence of polymerase, H-bonding is important
  - F-A is less stable than T-A
- H-bonding not as important to DNA polymerase:
  - F works like T
- DNA polymerase is enforcing the pairing
  - A and F do not selectively pair naturally

## Conclusion

F is the same shape as T

+

F does *not* do Watson-Crick pairing

+

F is almost as good as T during replication

→

Results support a model of replication that involves *shape* complementarity.