Part one
Primer design

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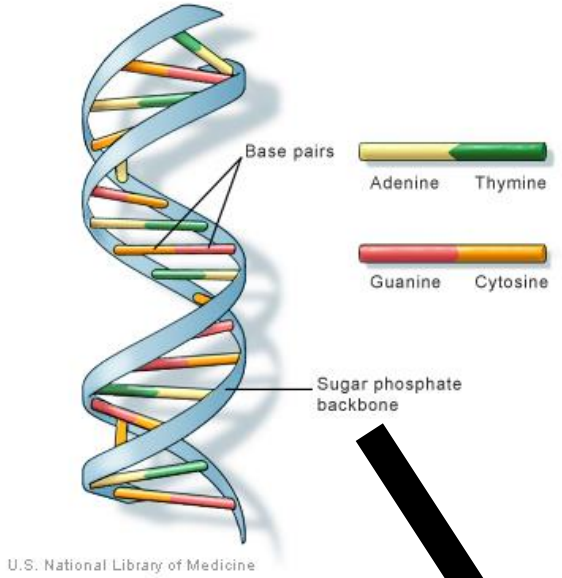
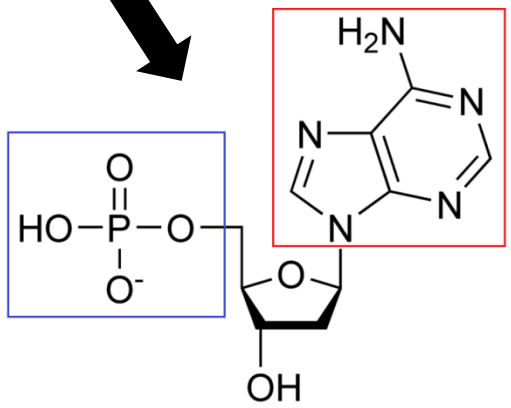
what is the purpose of designing primer?





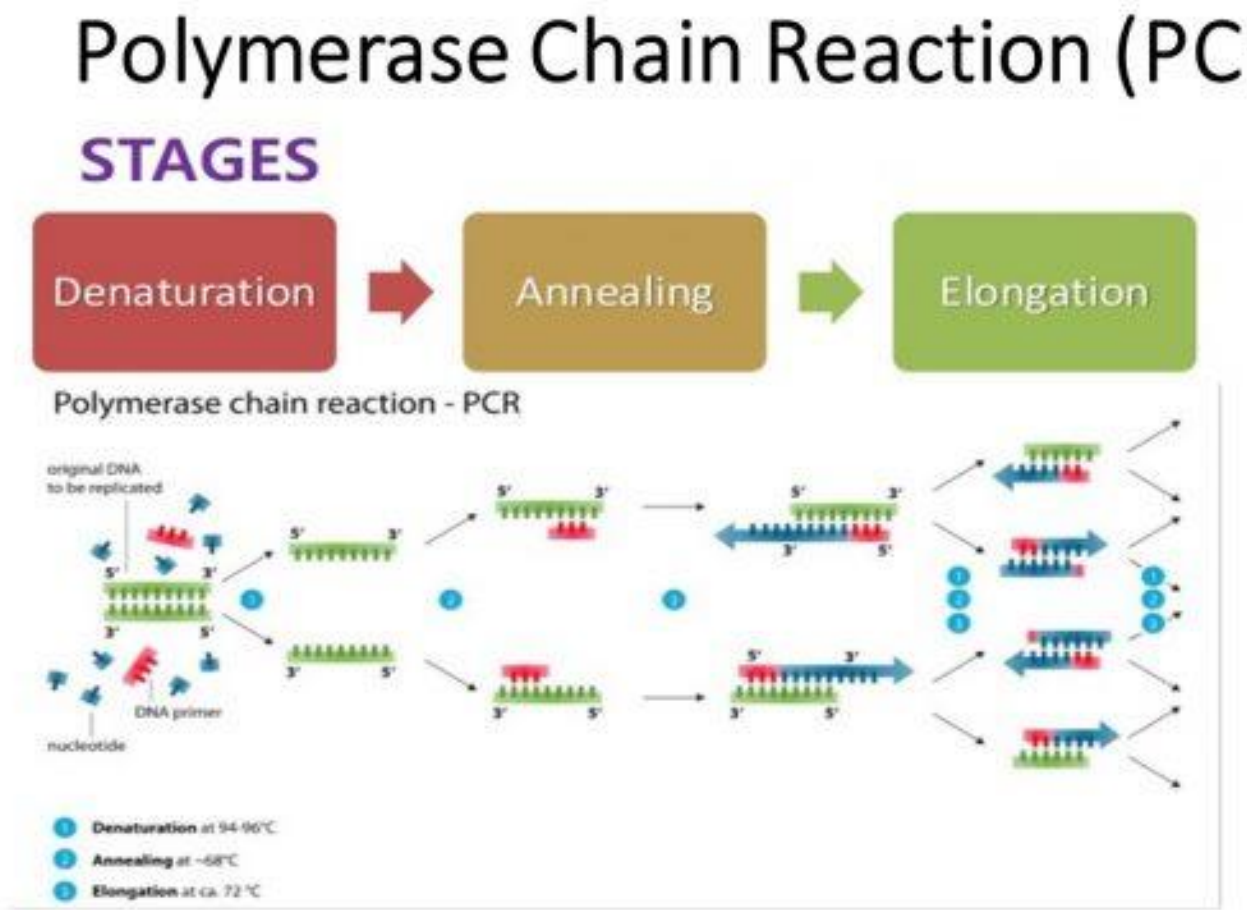


Table-10: The Reaction Mix (25 µl) for *CYP2D6* Gene.

Chemicals	Volume
Go Taq Green Master Mix	12.5 µl
Primer Forward	1.0 µl
Primer Reverse	1.0 µl
DNA	5.0 µl
D.W	5.5µl
Mineral oil	25.0µL

Table-11: PCR Condition for *CYP2D6* Gene.

Sr No.	Steps	Temperature	Time	No. Of cycles
I	Denaturation1	95.0C°	6min	1
II	Denaturation2	94.0C°	1min	35
III	Annealing	59.7C°	1min	
IV	Extension 1	72.0C°	1min	
V	Extension 2	72.0C°	5min	1

- Extension1 timedepend on amplified DNA fragments size

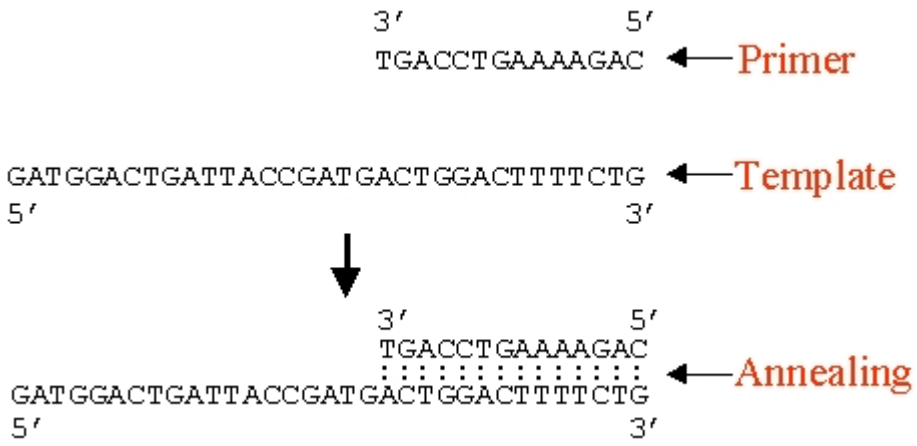
1Kb=1min

- Annealing temperature calculate using equation below

what is the purpose of designing primer?

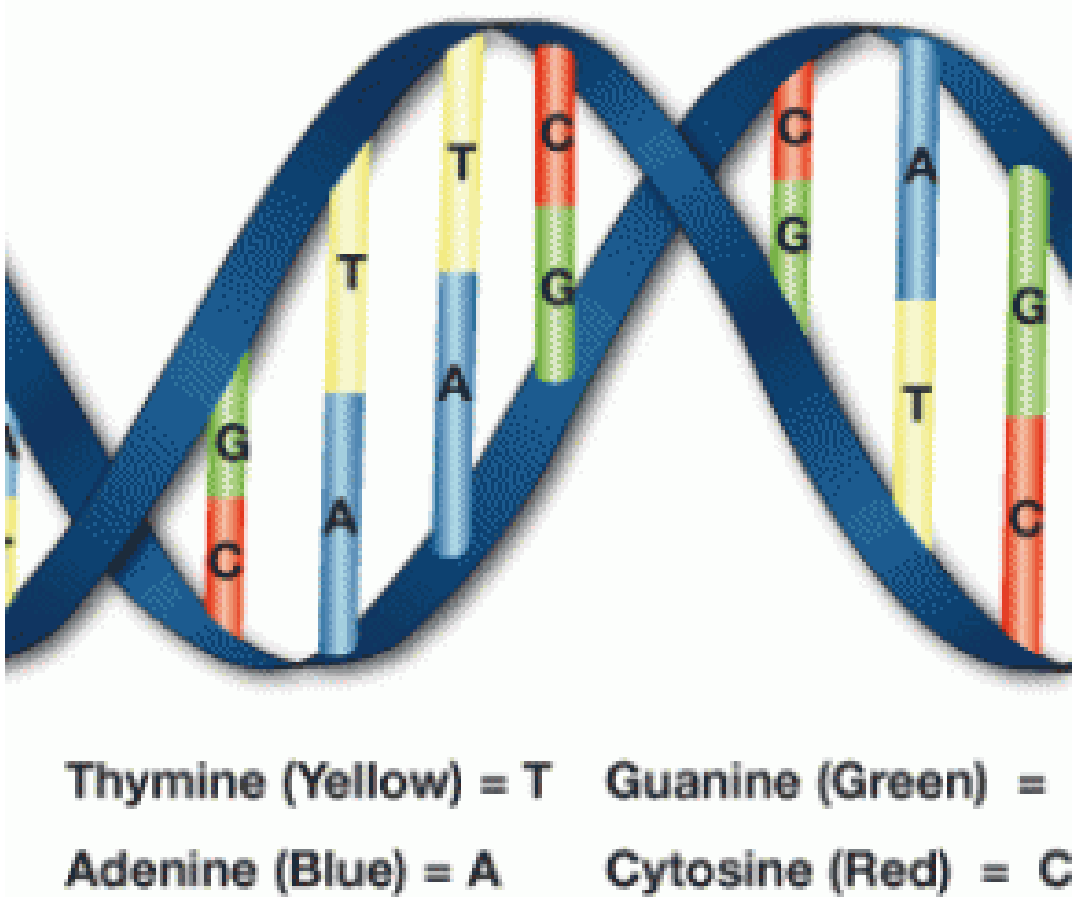
Primer are short sequence of single stranded DNA that are complementary to DNA sequences that flank the target region of interest.

The purpose of PCR primers is to provide a “free” 3'-OH group to which the DNA polymerase can add dNTPs.

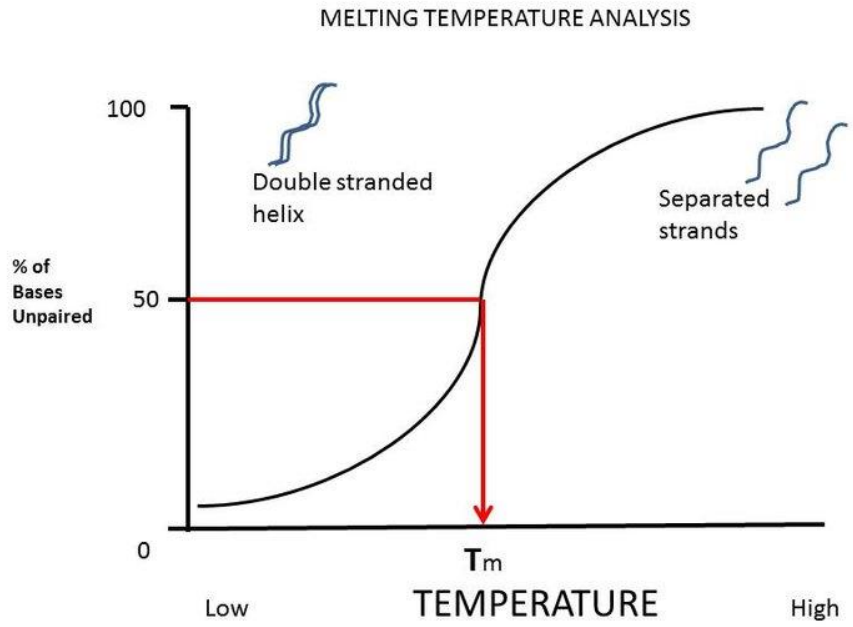


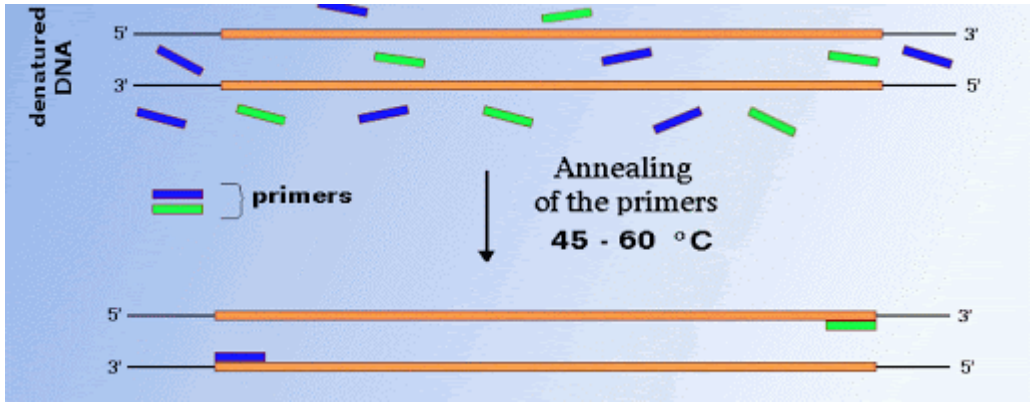
What shall we consider?

- Primer length: primer length should be between 18-24 bp.
- (G+C) ratio is between 50-60%.
- To increase primer efficiency, primers should be terminated in a G or C, or CG or GC.
- Tm (Melting temperature) is between 55-80°C.
- To avoid primer dimer, 3'-ends of primers should not be complementary.



- **Primer Melting Temperature**: **Primer Melting Temperature** (T_m) by **definition** is the **temperature** at which one half of the DNA duplex will dissociate to become single stranded and indicates the duplex stability.





he **annealing temperature** is the **temperature** used in the **annealing** step of a PCR reaction, which is highly dependent on the Tm of primers.

Steps to design primers

- Determine target sequence and find it online.
- Select a appropriate website or software for designing.
- Find annealing temperature (Ta) following:

Ta=Tm-3 or 5°C

Tm= (A+T) * 2 + (G+C) * 4

- https://www.thermofisher.com/

- **F- 5GAA CTC CCT GAA AAG CTA AAG C-3**
- **G=4, A=9, C=6, T=3**
- **Tm= (A+T) * 2 + (G+C) * 4**
- (9+3)*2+(4+6)*4=12*2+10*4=24+40=64
- **R- 5GTT GGG CTC AAA TAT ACG GTC G-3**
- **G=7, A= 5,C=4, T=6**
- **Tm= (A+T) * 2 + (G+C) * 4**
- (5+6)*2+(7+4)*4=22+44=66
- 66+64=130\2=65
- **Ta=Tm-3 or 5°C**
- 65-5=60