

## Primer design

Imagine you have the 5'-3' sequence of a gene and you wish to amplify a fragment of it.

5'-NNNNN**A**GAGACA GTGGGACCGT CTG ----- TGGG CTTGAGGATT CTAG**A**NNNNN-3'

**A** and **A** are the start and end of the fragment you wish to amplify.

----- : **means** the fragment is big enough so can't write the whole sequence.

(SENSE template)

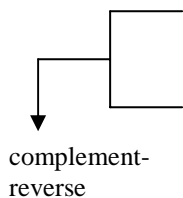
5'-NNNNN**A**GAGACAGTGGGACCGTCTG-----TGGACTTGAGGATTCTAG**A**NNNNN-3'

(ANTISENSE template)

3'-NNNNN**T**CTCTGTCCACCTGGCAGAC-----ACCTGAACTCCTAAGATCTC**N**NNNNN-5'

(ANTISENSE IN 5'-3' MODE):

5'-NNNNN**T**CTCTAGAACTCCTCAAGTCCA-----CAGACGGTCCCCTGTCTCT**N**NNNNN-3'



Some software like **DNassist** and **WinGene** can do "reverse-complement" in one click.

Thus, complement –reverse means: to have the sequence of the antisense in 5'-3' direction.

Now to design the primers using the 5'-3' (sense) strand:

One of the primers (the forward primer) will be directed from 5'-3' and anneal with the antisense template. This primer is written as same as the sense template nucleotide sequence.

Forward primer: **5'-NNNNNAGAGACAGTGGGACCGTCTG-3'**

NNNNN: the restriction sequence, GC clamp, ...etc.

The second primer (backward/antisense) will anneal with the sense template and thus it is 3'-5'.

Backward primer: **3'-ACCTGAACTCCTAAGATCTC**N**NNNNN-5'**

Usually both primers are written in 5'-3' formula. Thus, the backward primer can be written as: **5'-NNNNNCTCTAGAACTCCTCAAGTCCA-3'**

where NNNNN are the restriction sequence, extra nucleotides or GC clamps.



- (b) Avoid having  $\geq 3$  C or G (i.e. GGG) as this stabilizes the nonspecific annealing of the primers.
- (c) This end shouldn't be self-complementarily to avoid secondary structure (hairpin) formation or primer dimer formation.
- (d) Avoid **thymidine** at this end as it causes mispriming.

**(4) Tm is between 55-80C.**