BIOTECHNOLOGY
TOPIC – ANCHORED PCR

THIRD SEMESTER M.SC ZOOLOGY

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Anchored pcr

- It applied to double-stranded DNA fragments for which the sequence at only one end of the gene is known

- The technique allows amplification of a complete sequence of a gene when only the N-terminal sequence of a protein is known.

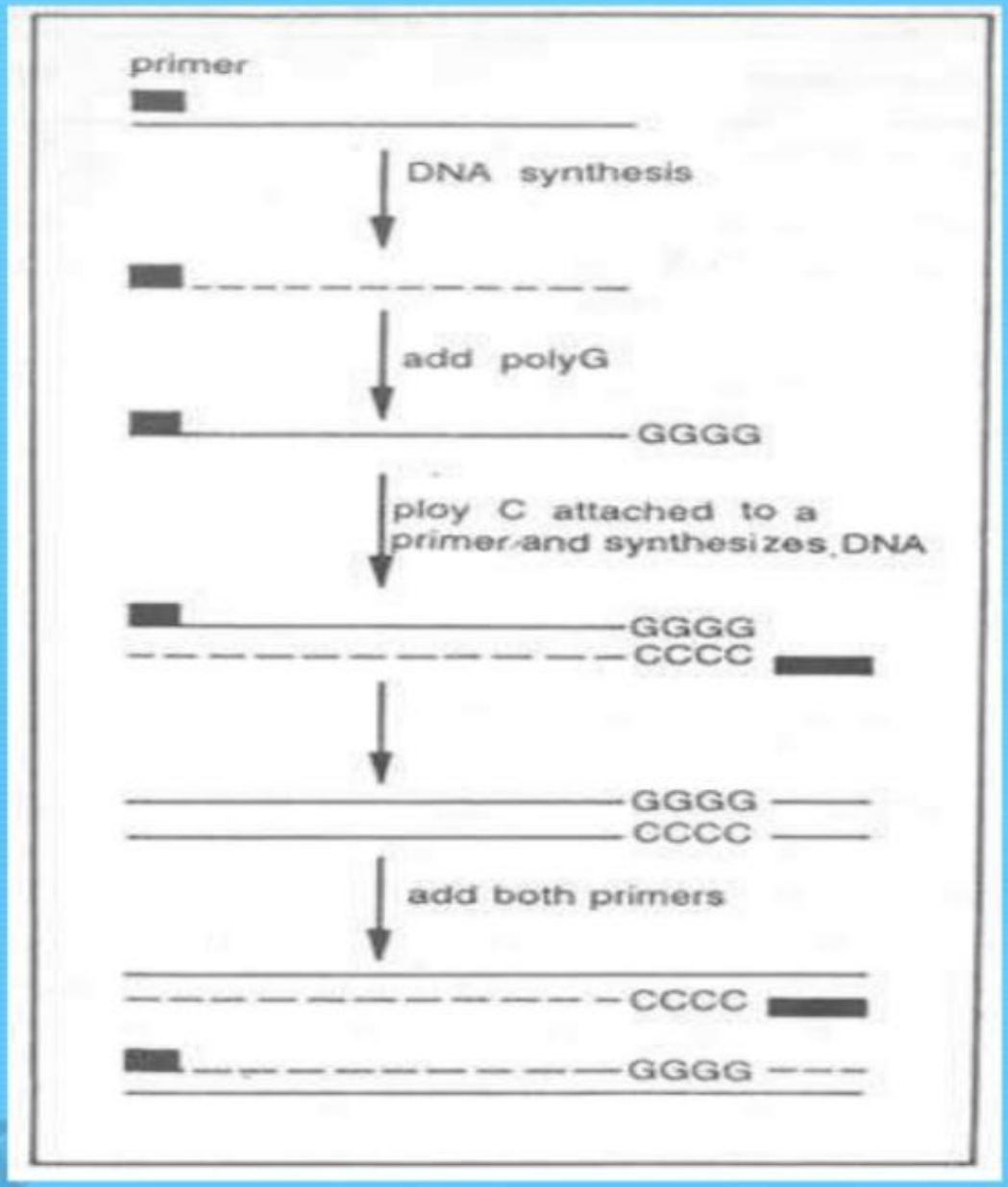
❑ Anchored PCR

-A small sequence of nucleotides can be attached or tagged to target DNA.

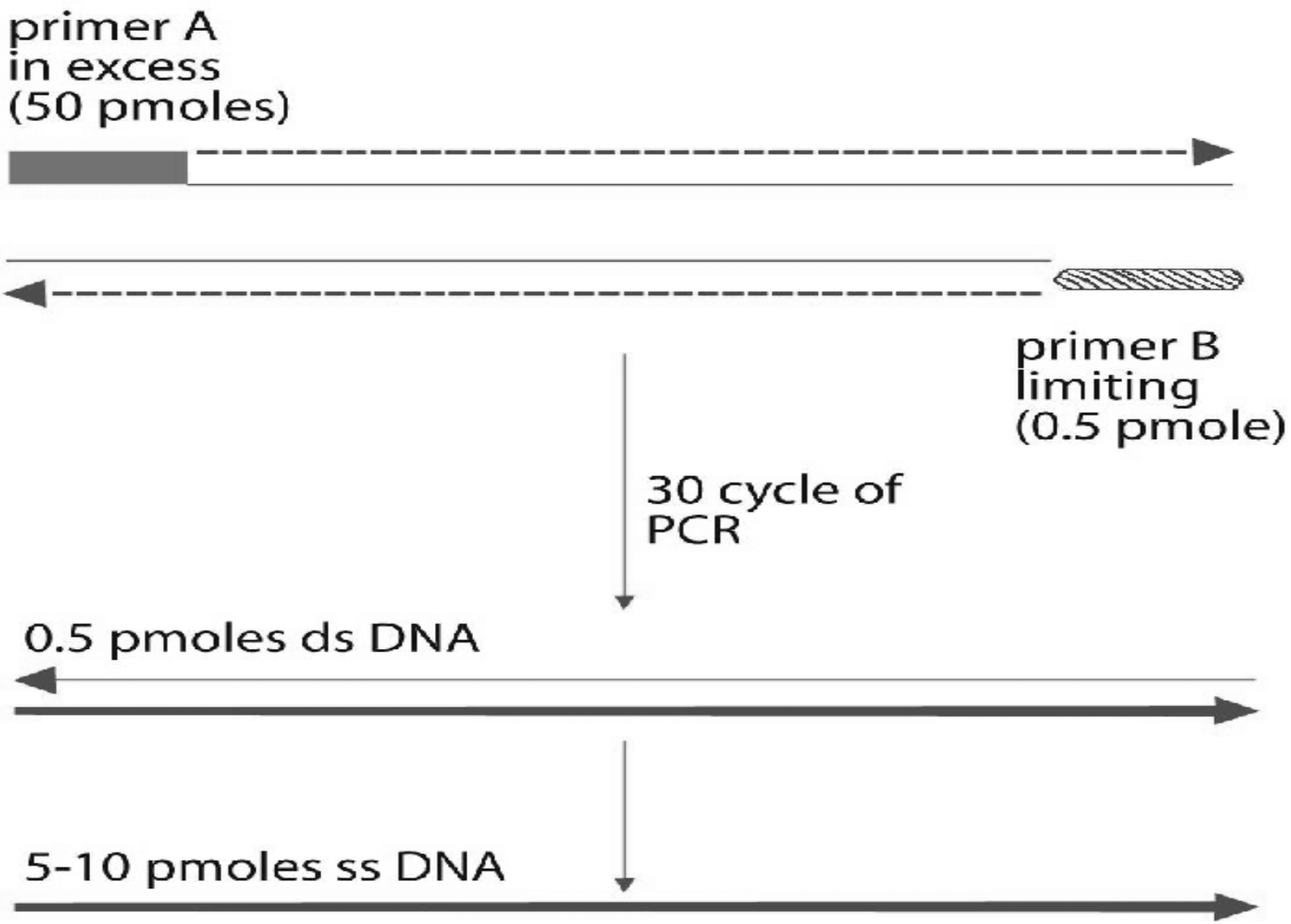
- The anchor is frequently a poly G to which a poly C primer is used.

Anchored PCR

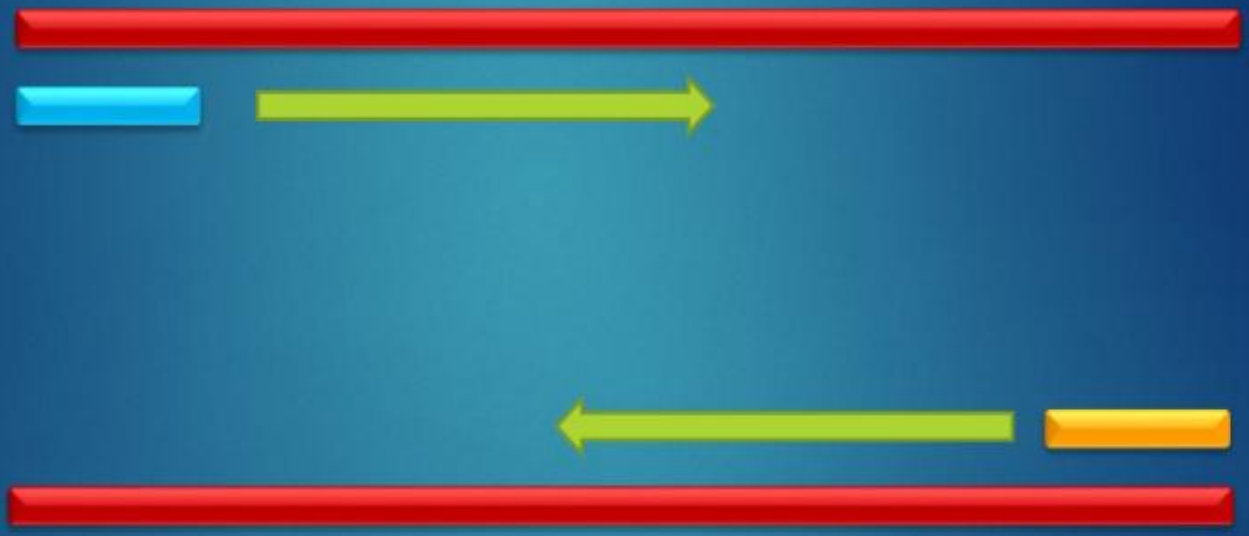
- In some type of PCR where only enough information to make a single primer is known, a known sequence is added to the end of the DNA by enzymatic addition of a polynucleotide stretch or by ligation of known sequence, and the second primer is designed by sequences of anchored DNA.
- This technique of amplification with single sided specificity has been known as one-sided PCR or anchored PCR.
- The anchored or single-sided PCR allows specific amplification of DNA where the 5' sequence of the molecule of interest is unknown.
- This approach is based on homopolymer tailing of cDNA catalyzed by the terminal deoxynucleotidyl transferase.
- The amplification is performed by using one primer specific for the molecule of interest (gene-specific primer), and a second primer containing a defined 'anchor' sequence attached to a homopolymer sequence complementary to the tail.


primer
DNA synthesis
add polyG
GGGG
ploy C attached to a primer and synthesizes DNA
GGGG
CCCC
GGGG
CCCC
add both primers
CCCC
GGGG

- Asymmetric PCR is used to preferentially amplify one strand of the original DNA more than the other.
- It finds use in some types of sequencing and hybridization probing where having only one of the two complementary stands is ideal.
- PCR is carried out as usual, but with a great excess of one primers for the chosen strand.


primer A
in excess
(50 pmoles)
primer B
limiting
(0.5 pmole)
30 cycle of
PCR
0.5 pmoles ds DNA
5-10 pmoles ss DNA

Asymmetric PCR: Synthesis of single strand DNA



Asymmetric PCR: Synthesis of single strand DNA

In a regular PCR the same amount of forward and reverse primers is added

Asymmetric PCR: Synthesis of single strand DNA

In an asymmetric PCR the one of the primers is largely in excess compared to the other

Asymmetric PCR: Synthesis of single strand DNA