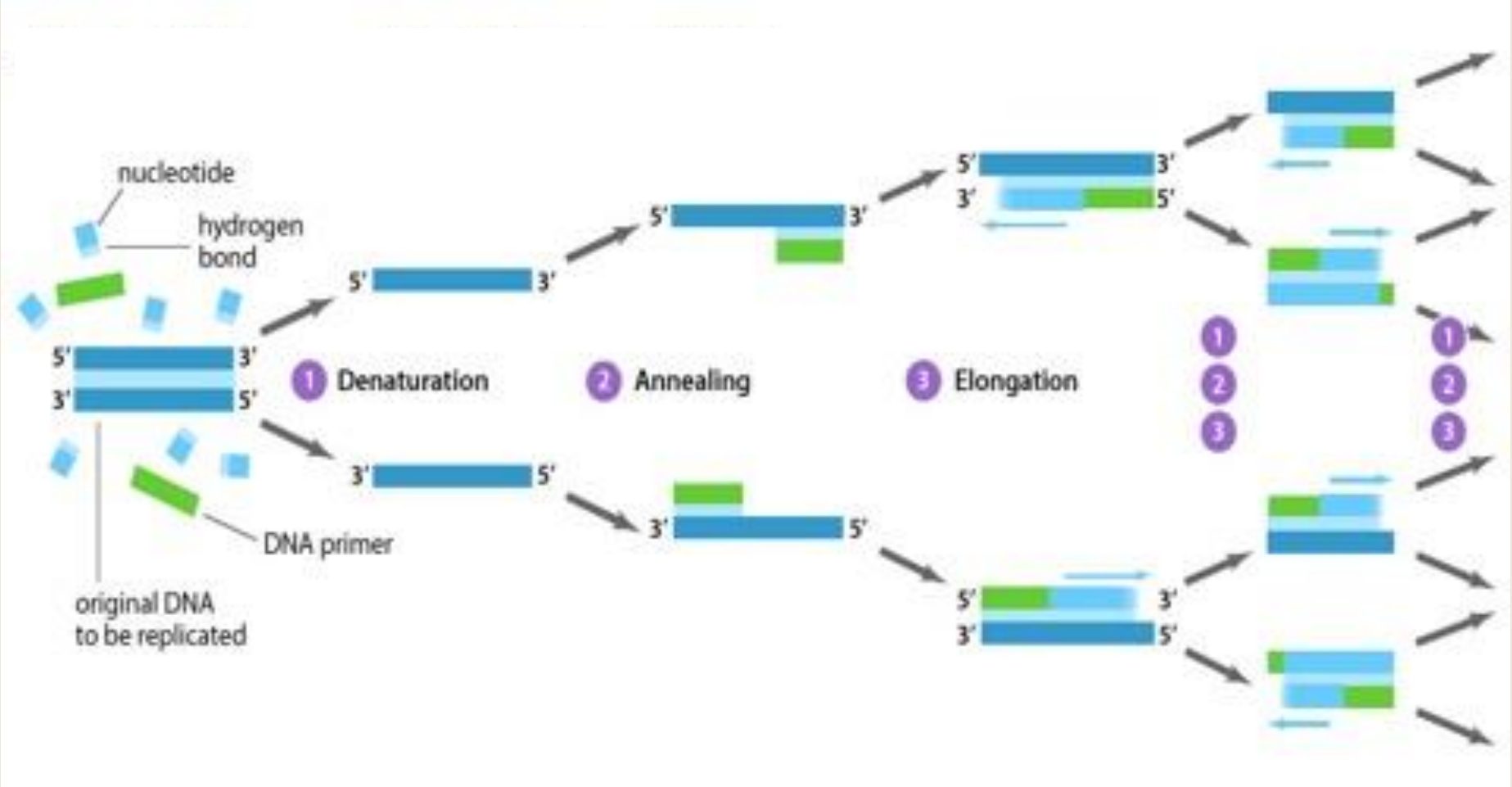
BACTERIAL IDENTIFICATION BASED ON UNIVERSAL GENE AMPLIFICATION AND SEQUENCING

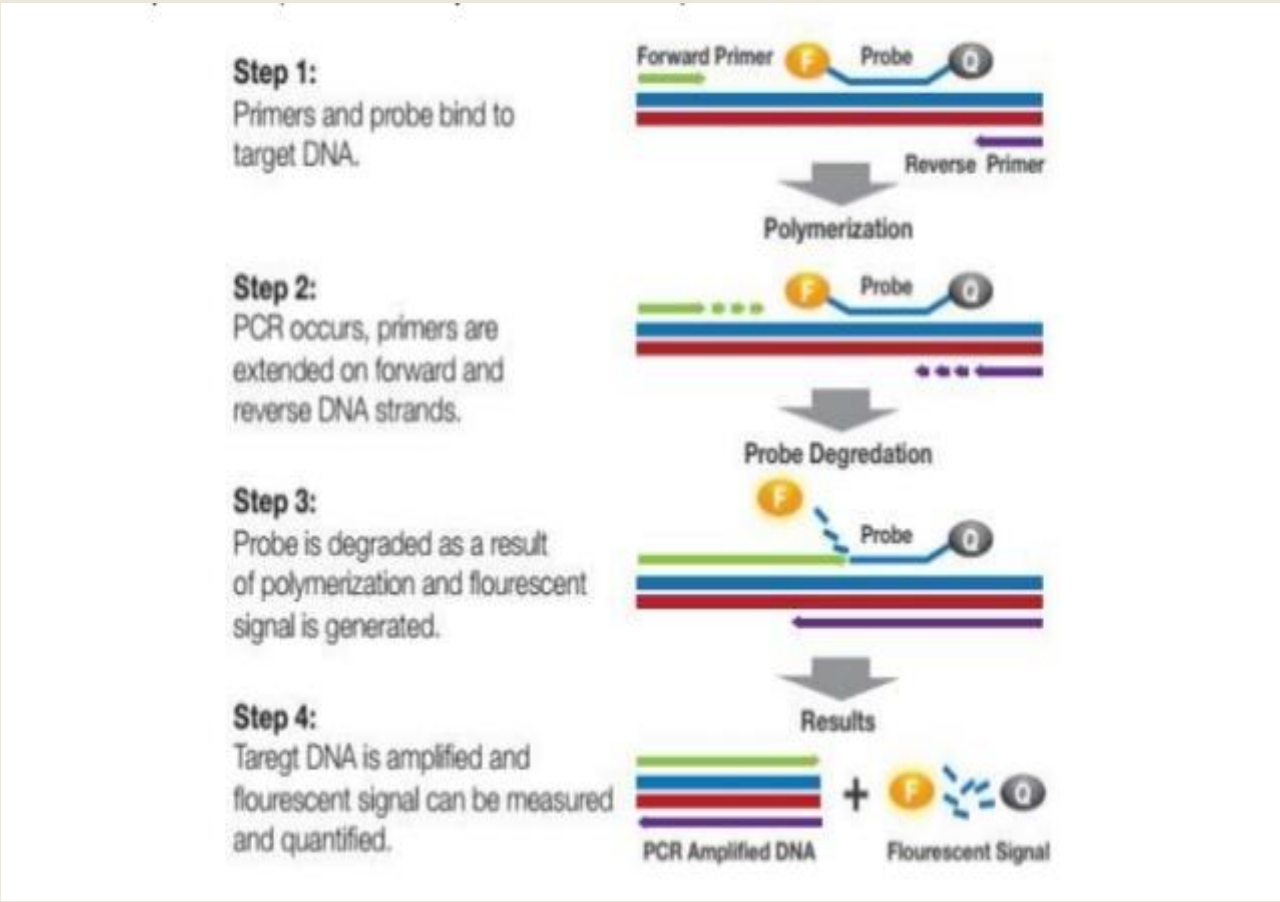
PCR and Its Variations

1- Polymerase Chain Reaction Reaction

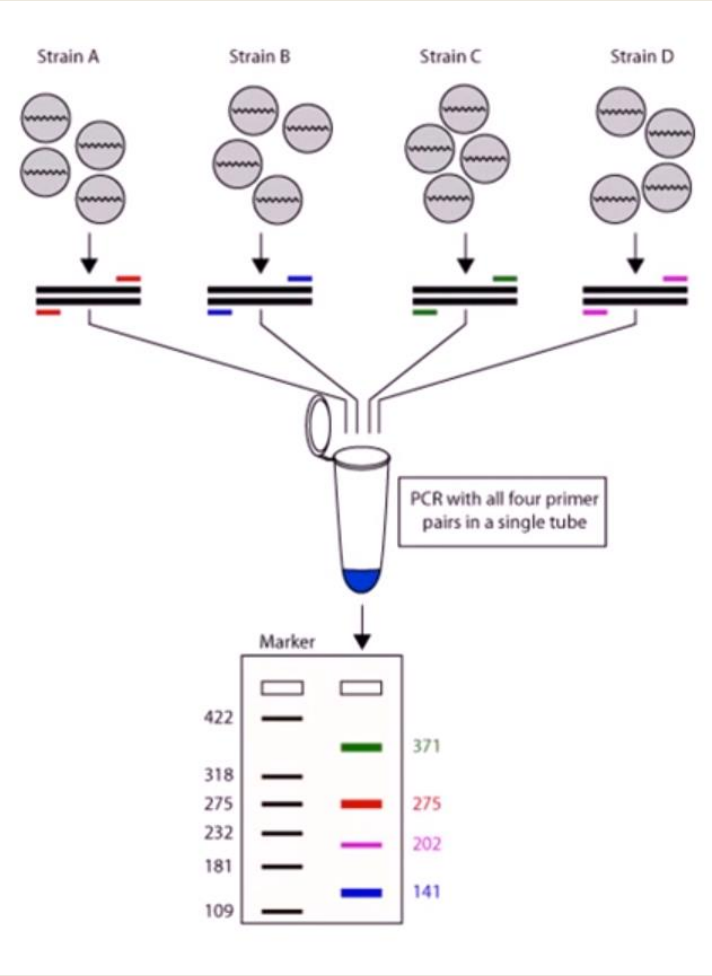


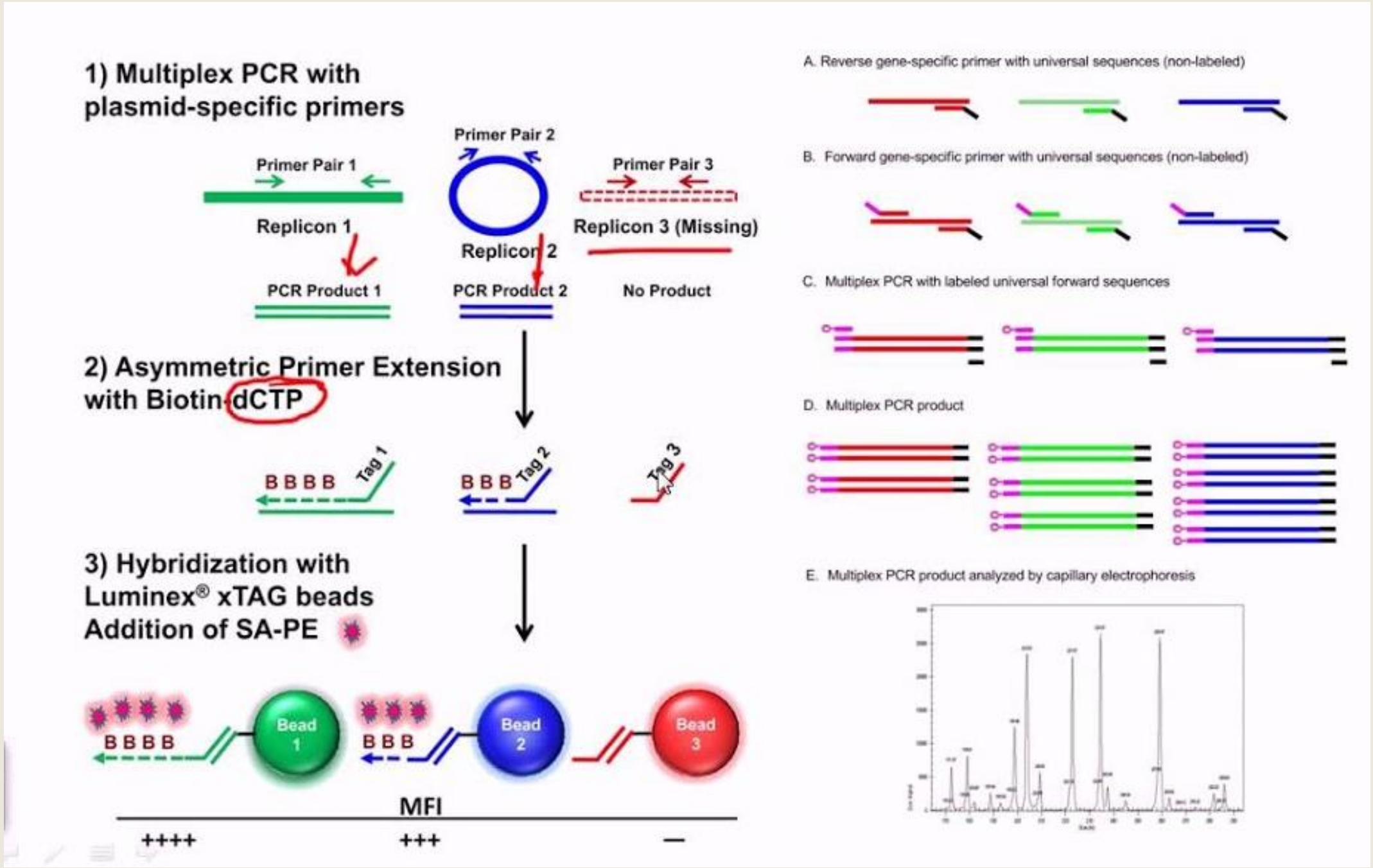
2- Quantitative Polymerase Chain Reaction

- (qPCR), called real-time **PCR**



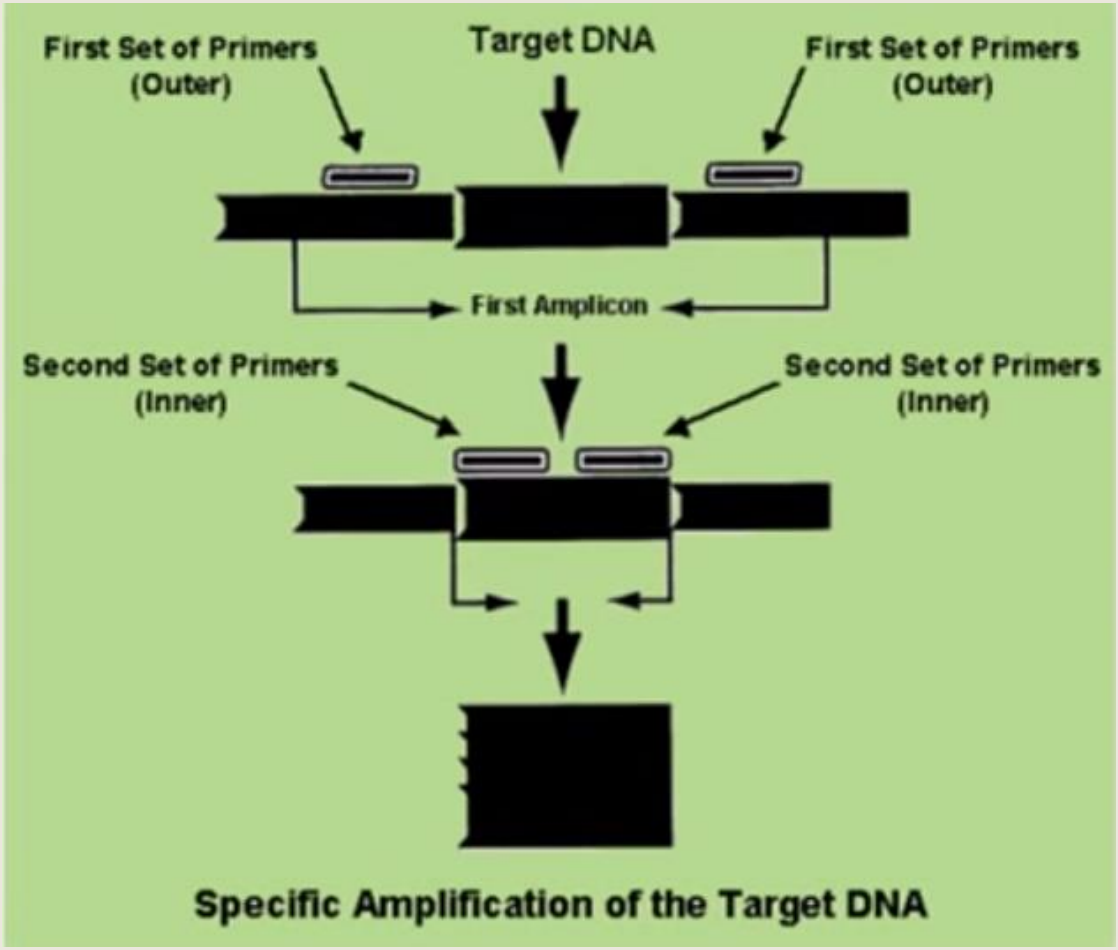
3- Multiplex Polymerase Chain Reaction




1) Multiplex PCR with plasmid-specific primers
Primer Pair 1
Primer Pair 2
Primer Pair 3
Replicon 1
Replicon 2
Replicon 3 (Missing)
PCR Product 1
PCR Product 2
No Product
2) Asymmetric Primer Extension with Biotin-dCTP
BBBB
Tag 1
BBB
Tag 2
Tag 3
3) Hybridization with Luminex® xTAG beads
Addition of SA-PE
BBBB
Bead 1
BBB
Bead 2
Bead 3
MFI
++++
+++
—
A. Reverse gene-specific primer with universal sequences (non-labeled)
B. Forward gene-specific primer with universal sequences (non-labeled)
C. Multiplex PCR with labeled universal forward sequences
D. Multiplex PCR product
E. Multiplex PCR product analyzed by capillary electrophoresis

4- Nest Polymerase Chain Reaction

- Very low probability of nonspecific amplification
- Primers – high specificity
- Two pairs (instead of one pair) of PCR primers are used to amplify a fragment.
- First pair -amplify a fragment similar to a standard PCR.
- Second pair of primers-nested primers



5- variable number tandem repeat

VARIABLE NUMBER TANDEM REPEAT

loci are chromosomal regions in which a short DNA sequence is repeated a variable number of times end-to-end at a single location. These can be found on many chromosomes, and often show variations in length between individuals. Each variant acts as an inherited allele, allowing them to be used for personal or parental identification.