GENETIC ENGINEERING

INTRODUCTION

- The deliberate modification of an organism’ s genetic information by directly changing the sequence of nucleic acids in its genome is called ***genetic engineering .***
- Accompanied by a collection of methods known as ***recombinant DNA technology***

- First the DNA responsible for a particular phenotype is identified and isolated
- After purification, the genes are fused with another piece of DNA called a cloning vector to form recombinant DNA.
- Then it is inserted into a suitable vector.

HISTORY

- Genetic engineering became possible after the discovery of restriction enzymes and reverse transcriptase, and the development of essential methods in nucleic acid chemistry such as southern blotting tecniques.
  - Restriction enzymes discovered in the late 60s by Werner Arber and Hamilton Smith
  - In 1972 David Jackson, Robert Symons, and Paul Berg successfully generated recombinant DNA Molecules
  - Southern blotting(1975)

RECOMBINANT DNA TECHNOLOGY

The procedure of recombinant DNA technology involves :

1. Treatment with restriction enzyme
2. Southern blot
3. Recombination with a vector
4. Introduction of vector into bacteria
5. cloning

1. ***Treatment with restriction enzymes***–The DNA is extracted from the microorganism and then it is cleaved by enzymes called restriction endonucleases to form DNA fragments.

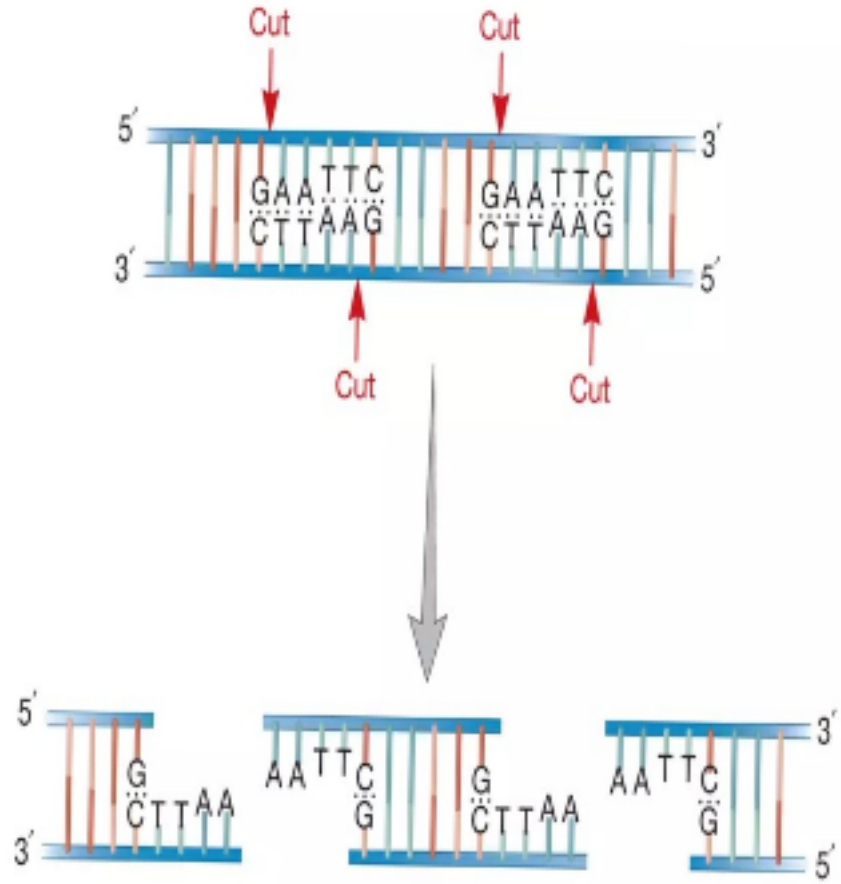


Figure 14.3 Restriction Endonuclease Action. The cleavage catalyzed by the restriction endonuclease *EcoRI*. The enzyme makes staggered cuts on the two DNA strands to form sticky ends.

2.Southern blot: The fragment containing desired gene is isolated from the cleaved DNA fragments.

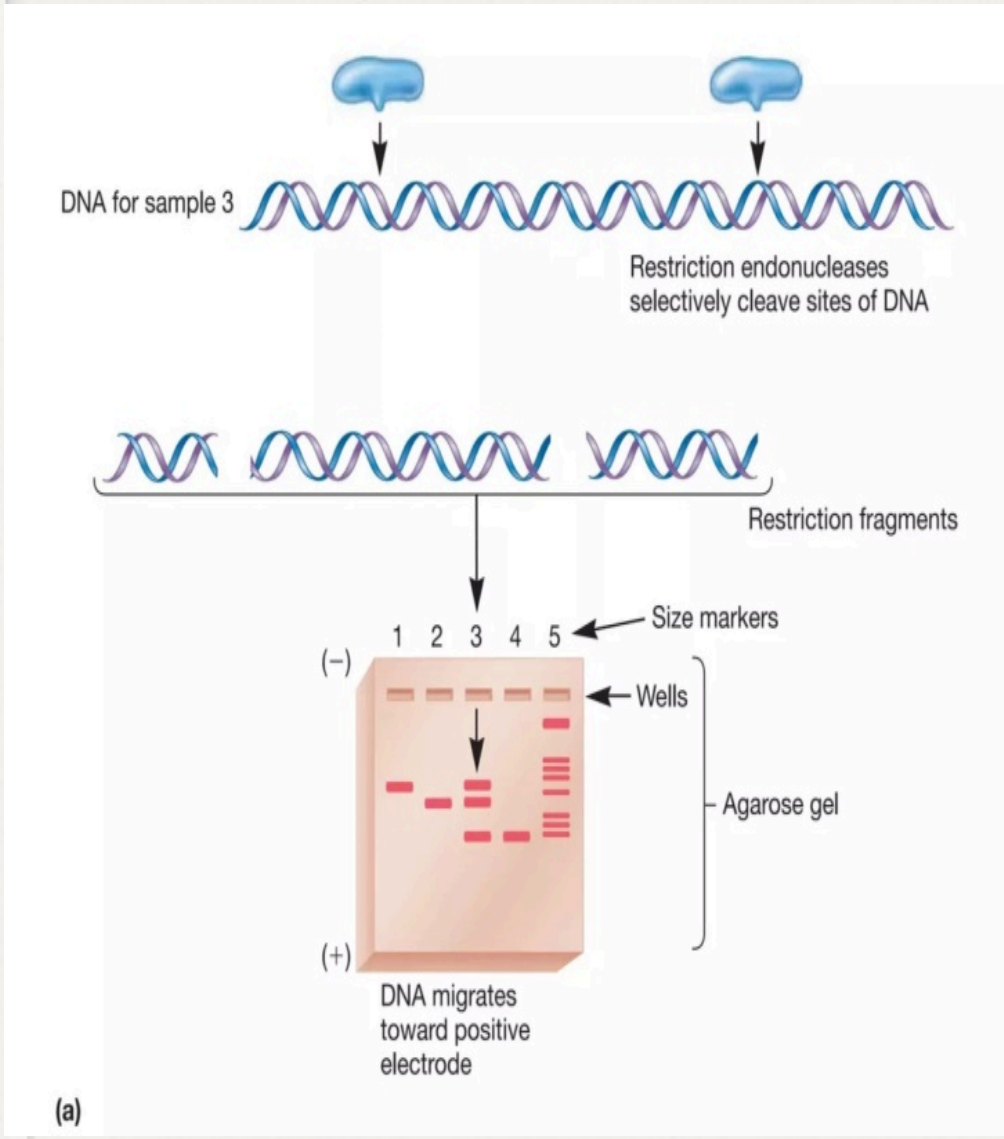
Done by electrophoresis

Electrophoresis: DNA fragments separate according to their size in agar gel elecctrophoresis

.Separated fragments are then transferred to nitrocellulose membrane

.The desired DNA fragment is detected by adding a special DNA probe, complementary to gene of interest

.The band containing desired gene is isolated by DNA extraction.



PCR

- POLYMERASE CHAIN REACTION
- Used to amplify a single or few copies of DNA to generate millions of copies of DNA

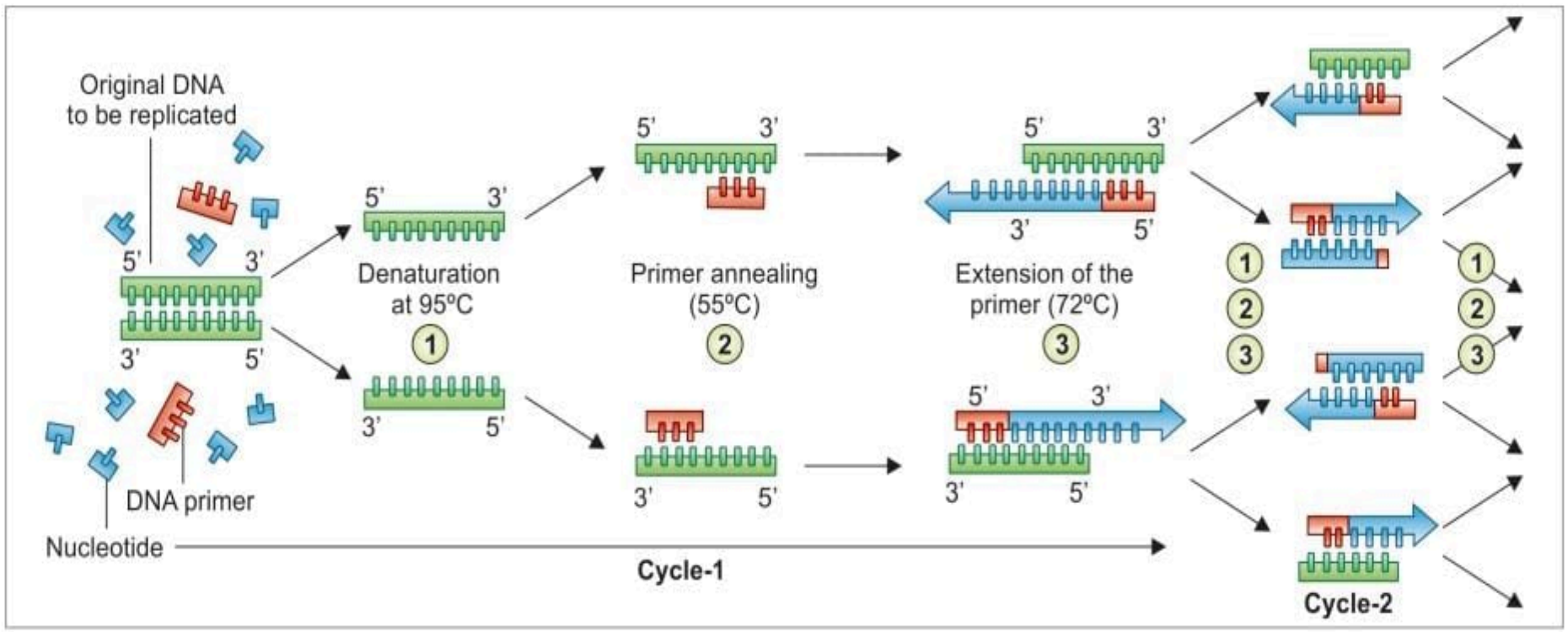


Fig. 3.3.29: Polymerase chain reaction cycle—3 basic steps of amplification.

- Carried out in thermocycler

Includes 3 steps:

a. *Denaturation* - seperation of dsDNa into two seperate single stands(95° C)

b. *Primer annealing* - primer anneals to the complementary site on target ss DNA(55° C)

c. Extension- catalyses by Taq Polymerase which keeps on adding free nucleotides to the growing end of primer

3. Recombination with a vector- the isolated d-DNA is annealed with a vector by DNA ligase.

. The most common approach to cloning is to digest both vector and DNA to be inserted with same restriction enzymes or enzymes , so that compatible sticky ends are generated.

First bacterial host- pSC101

.The vector and DNA to be clones are then incubated invitro in the presence of DNA ligase, which catalyses covalent insertion of the DNA fragment into the vector

. There are four types of cloning vectors- plasmids , pages , cosmids, artificial chromosomes

4. Introduction of vector into bacteria– the vector is then introduced to bacteria by transfromation (mainly) and transduction(rarely).

. A variety of techniques are used to introduce DNA to host cells using electroporation, micro injection, and gene gun

5. Expression of foreign gene in products-culture of bacteria containing desired gene followed by expression of gene products .

Many useful products such as somatostatin hormone, have been synthesized by recombinant DNA technology,

APPLICATIONS

- Production of vaccines
- Production of medically useful proteins insulin, somatostatin, human growth hormone , and some interferons
- Production of antigens used in diagnostic kits
- Production of proteins used in therapy
- Transgenic animals
- Gene therapy

BLOTTING TECHNIQUES

- Refers to the method of transferring DNA, RNA, or proteins from the gel to carrier, followed by their detection using specific nucleic acid probes or enzyme immunossay.

1. Southern blot- used to detect DNA
2. Northen blot- used to detect RNA
3. Western blot- used to detect proteins