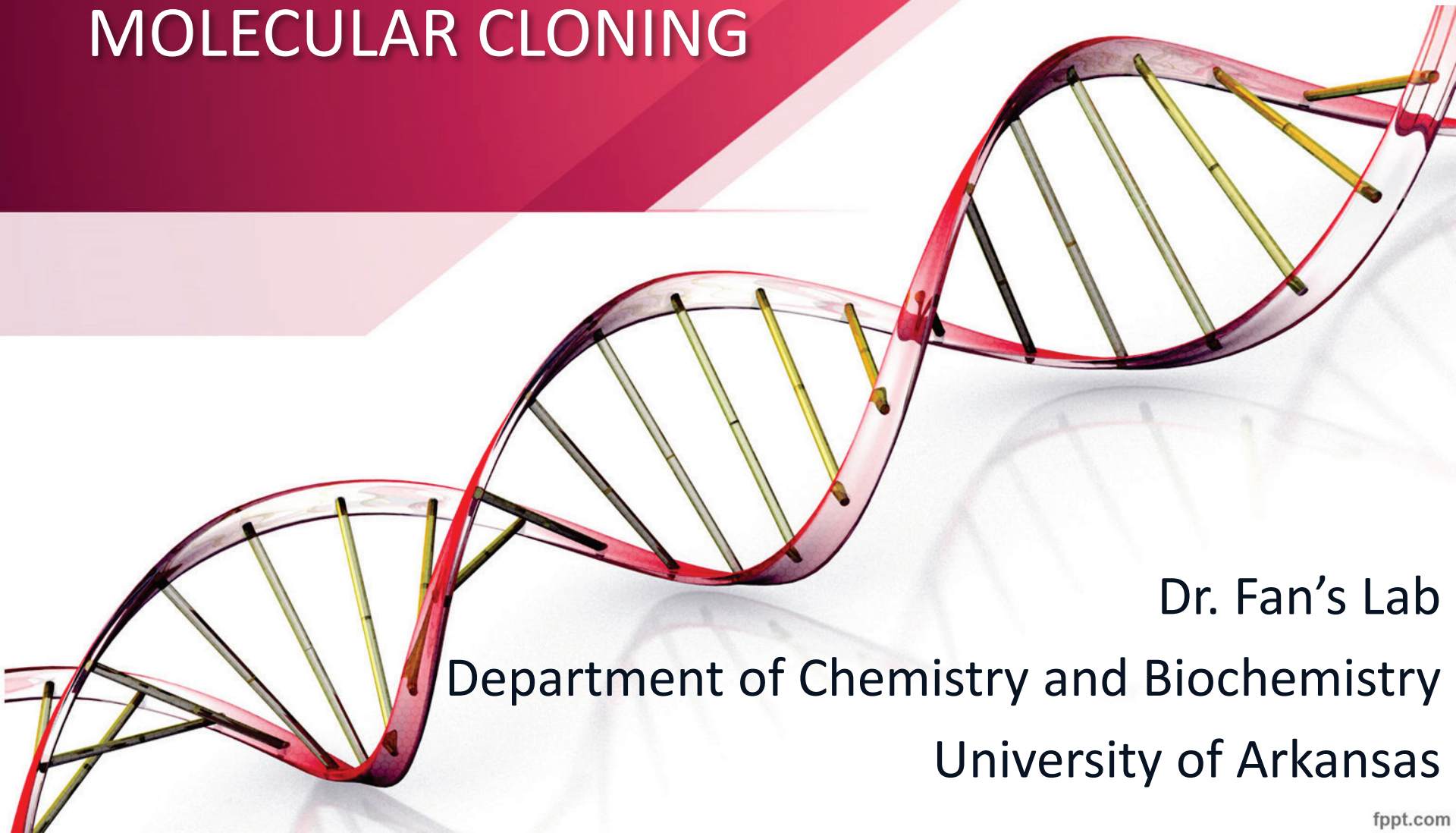
MOLECULAR CLONING



Dr. Fan's Lab

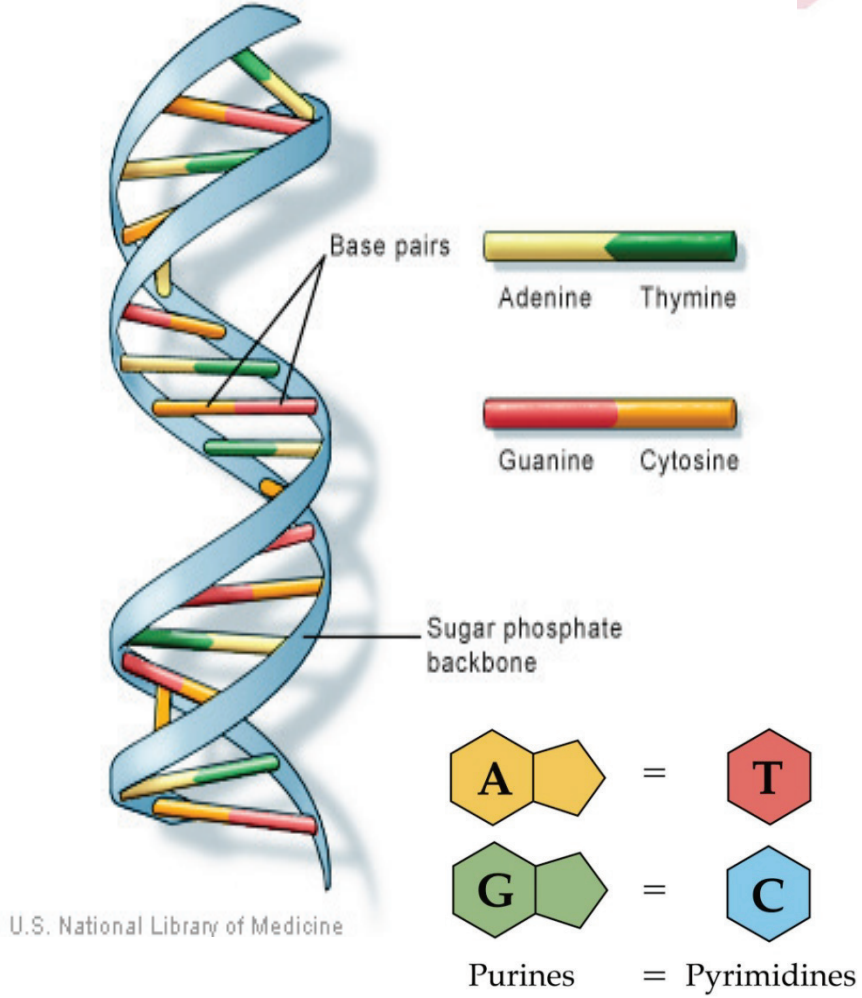
Department of Chemistry and Biochemistry

University of Arkansas

What is the genetic material in living beings called?

What is this genetic material made up of?

DNA – the backbone of life



- Carries genetic information for all the processes in the body.
- Double stranded antiparallel.
- Consists of deoxyribose sugar and phosphate framework.
- Nitrogen containing nucleobases called
  - adenine (A)
  - guanine (G)

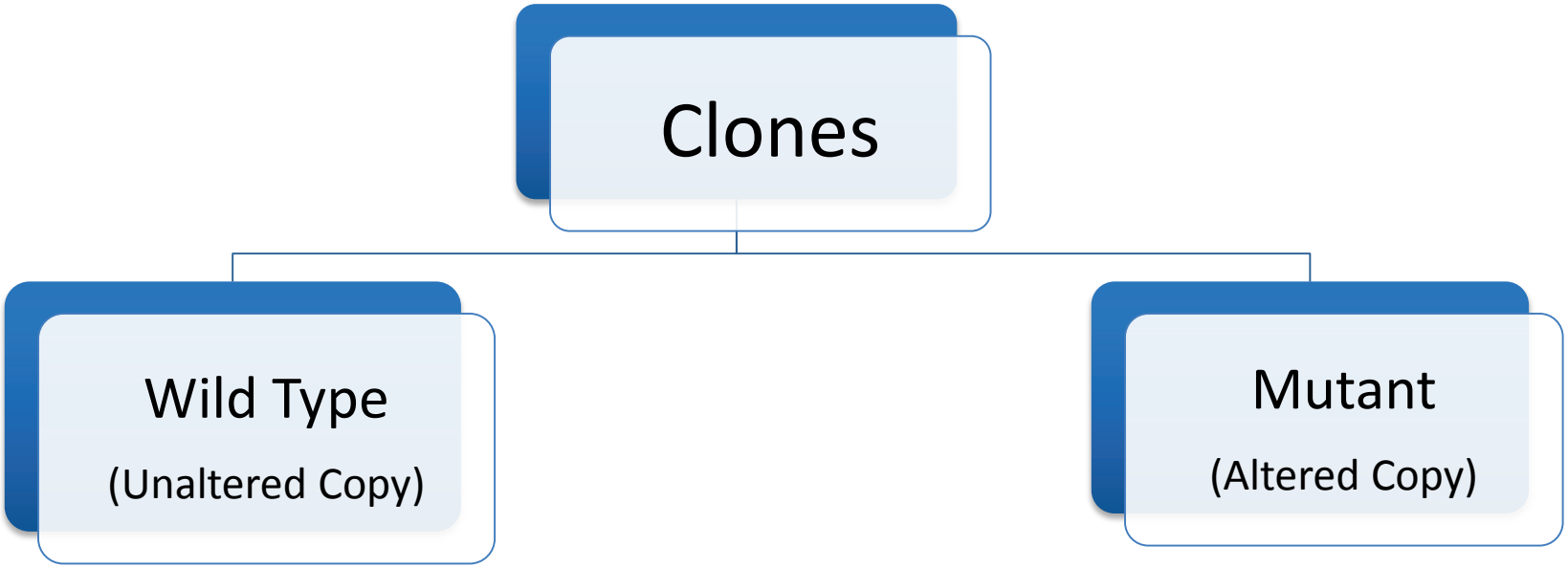
  } Purines

  - cytosine (C)
  - thymine (T)

  } Pyrimidines
- Base Pairing with hydrogen bonds:
  - A ═ T
  - C ═ G

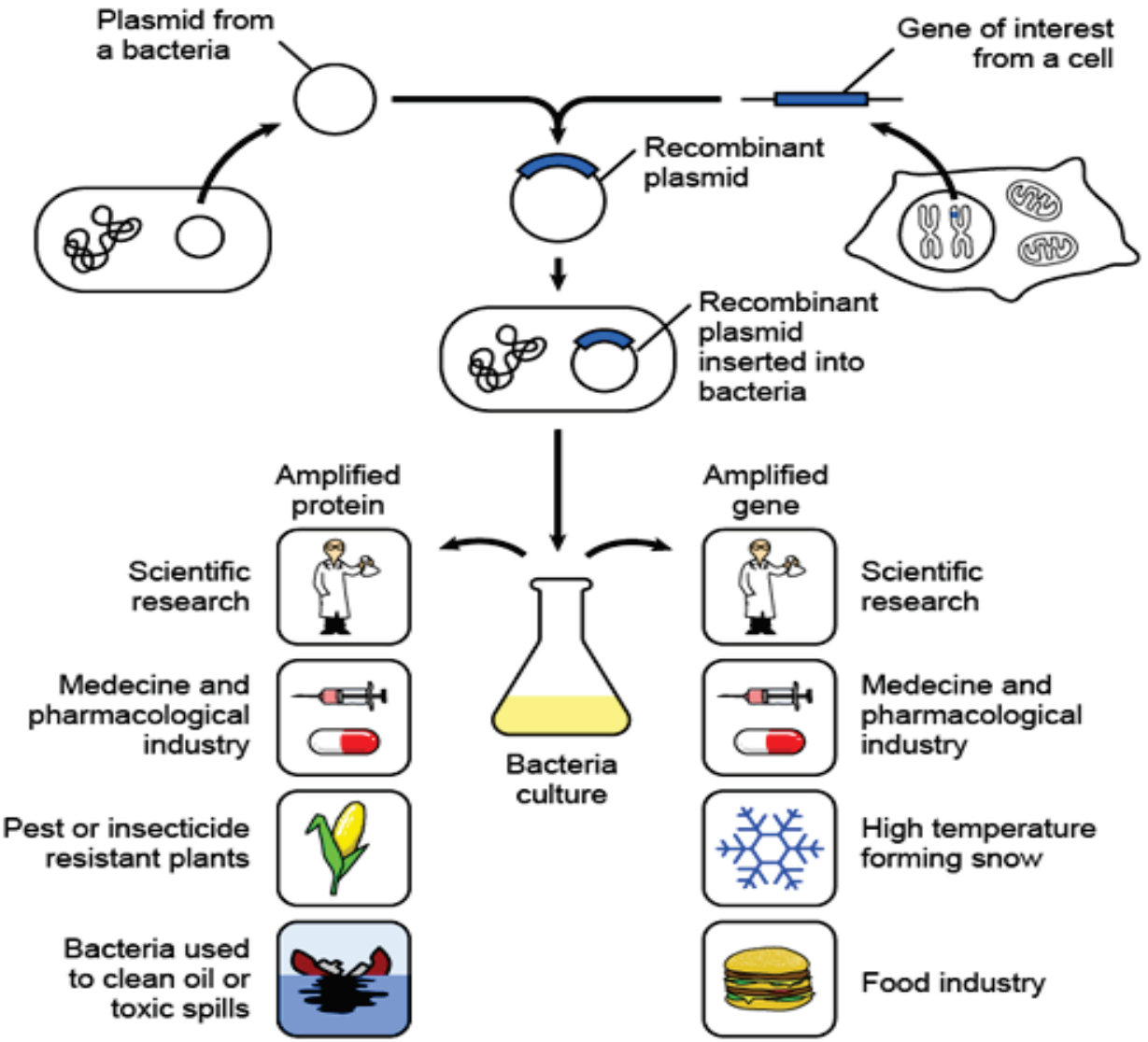
What is “Cloning”?

- Clone? Identical to the original source
- To clone means to replicate or make copies of the source



- rDNA (recombinant DNA) Technology: Manipulation of DNA

Recombinant DNA Technology



Few Methods of Gene Manipulation

- **Microprojectile Bombardment:** naked DNA could be delivered to plant cells by “shooting” them with microscopic pellets.
- **Transfection:** microinjection of DNA into the nucleus of anchored cells.
- **Transposons/Transposable Elements:** mobile elements present in the DNA.
- **Somatic Hybridization:** two protoplasts of plant cells fuse, the resulting somatic hybrid contains the genetic material from both plant sources.

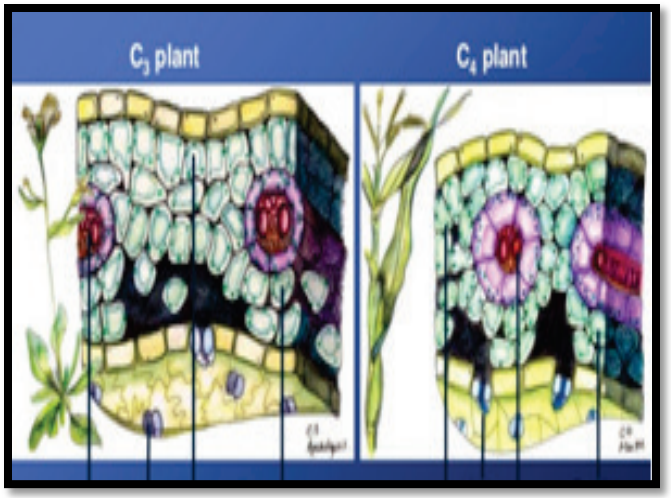
Applications of rDNA technology

AGRICULTURE

Transgenic Plants

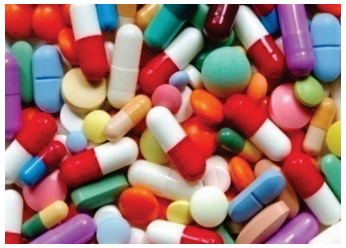
Root Nodules in Cereal Plants

Increase of Photosynthetic rate in plants

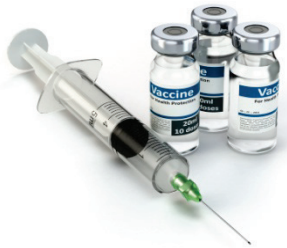


Applications of rDNA technology

Gene Therapy



Antibiotics

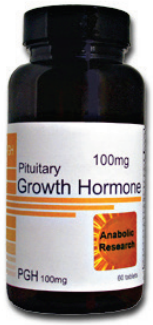


Vaccines

Transgenic animals

MEDICINE

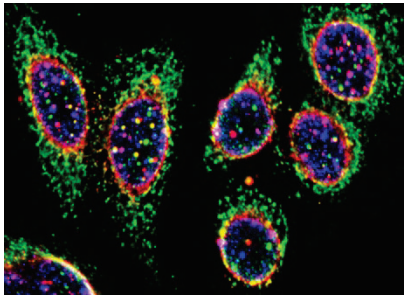
Hormones



Interferon

Enzymes

Diagnosis



MOLECULAR CLONING

- BASICS OF MOLECULAR CLONING:

- Target DNA segment
- Vector – Plasmid
- Restriction
- Polymerase Chain Reaction
- Transformation
- Selection
- Confirmation of results

STEPS INVOLVED IN MOLECULAR CLONING

1. To amplify the green fluorescence gene by PCR using a plasmid
2. To verify the plasmid using agarose gel electrophoresis
3. To transform the competent cells with the green fluorescence plasmid
4. To culture the cells on an agar plate and to grow them overnight
5. To check the plates and confirm the green fluorescence

TARGET DNA & VECTOR DNA

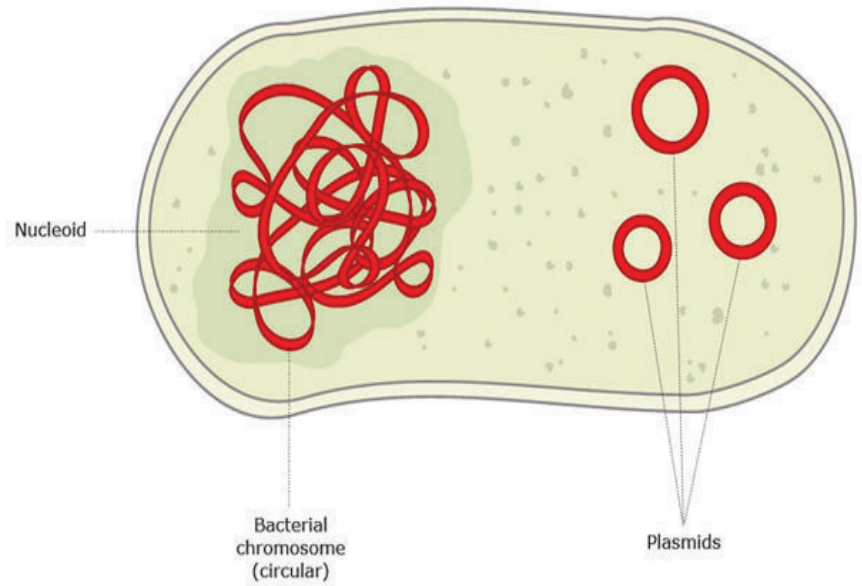
TARGET DNA:

- The target DNA is the DNA of interest
- The DNA to be amplified

VECTOR DNA:

- To carry the target DNA
- To maintain the target DNA in the host
- Passed into daughter cells during cell division

PLASMID



- Extra chromosomal DNA
- Circular in shape
- Independent in replicating
- Can be transferred to other cells
- Can be integrated into the chromosome
- Have antibiotic resistance

Polymerase Chain Reaction

- A process amplify a single copy of DNA into millions of copies
- Requires:
  - Primers
  - Template
  - Polymerase
  - Substrate
  - Thermo cycler

Constituents of PCR

- **Primers:** Small section of DNA nucleotides which bind to the single-stranded DNA template during PCR.
- **Template:** That particular portion of a DNA molecule which is copied in PCR.
- **Polymerase:** The enzyme which catalyzes the reaction of adding new DNA bases to a growing DNA strand.
- **Reaction buffer:** A reaction buffer is used to provide a stable pH. It may also contain magnesium chloride

Agarose Gel Electrophoresis

- Provides a meshwork and controls the movement of the DNA based on the size (small fragments move faster).
- Ethidium bromide is added to the gel which acts as an intercalating agent.
- Running buffer increases ionic strength and maintains pH.
- Loading dye (bromophenol blue and xylene cyanol) provides color and the ficoll in it increases the density of the sample.
- Power supply: 130V for 20minutes.

Transformation

- **Competent Cells:** cell membranes are disrupted and can take up DNA from their surrounding environment and integrate it into their own chromosomes by recombination.
- **Heat shock:** fluidity of the membrane changes, DNA can then enter the bacteria at an efficient rate.
- Incubation for 30 minutes: this is done so that the antibiotic resistance gene in the plasmid is activated and thus the cells become capable of growing on agar plate with antibiotic.

Thank you