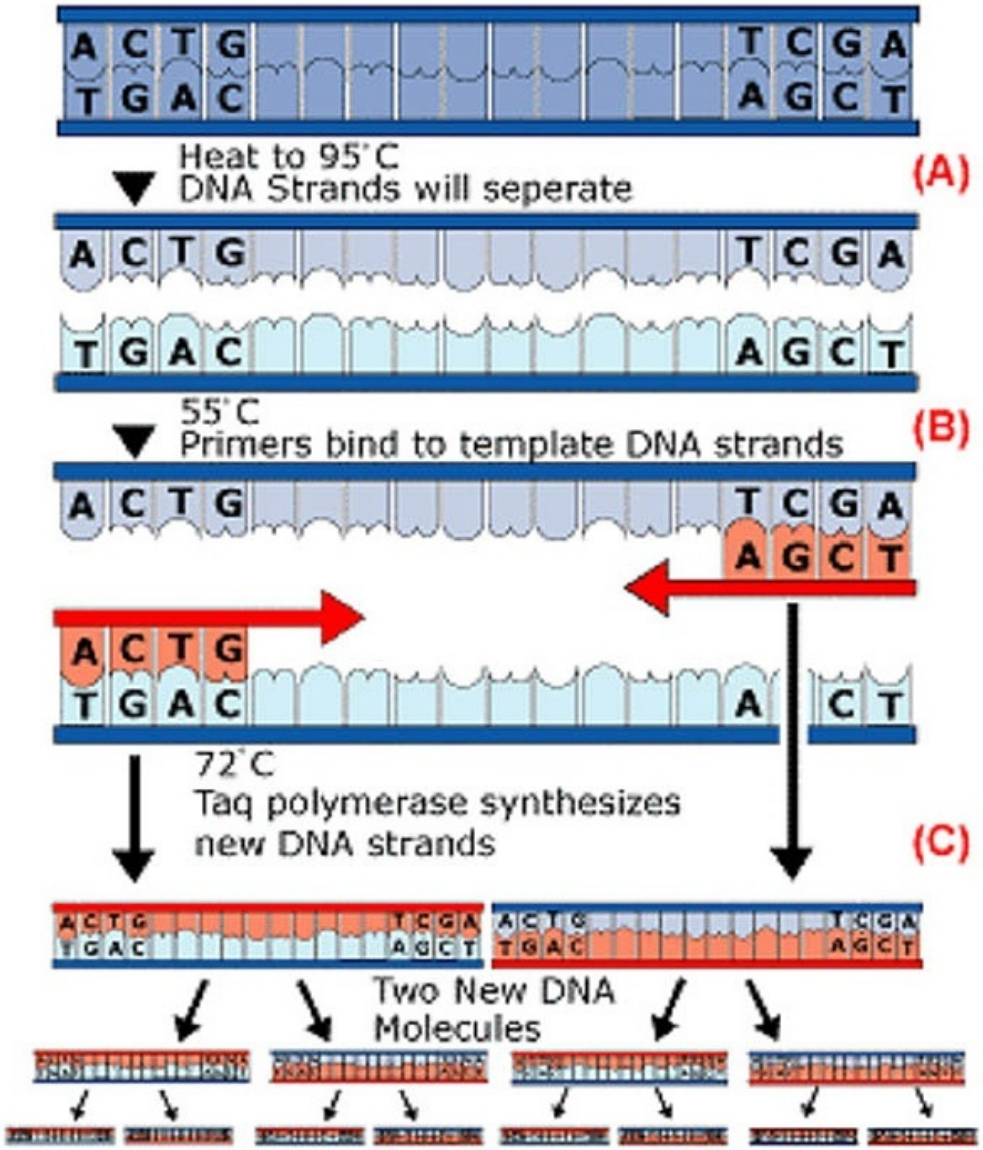
Chapter 4

Recombinant DNA Technology Tools and Genomics

Polymerase chain reaction (PCR)

- To amplify a single or a few copies of DNA
- Generated thousands to millions of copies of a particular DNA sequence
- DNA as a template for replication

- Use primer: short DNA fragments containing sequences complementary to the target region along with a DNA polymerase
- PCR mixture (DNA template, dNTP, Buffer, $MgCl_2$, primer Forward and primer Reverse, taq polymerase enzyme and water


Heat to 95°C
DNA Strands will seperate
(A)
55°C
Primers bind to template DNA strands
(B)
72°C
Taq polymerase synthesizes
new DNA strands
(C)
Two New DNA
Molecules

Application of PCR

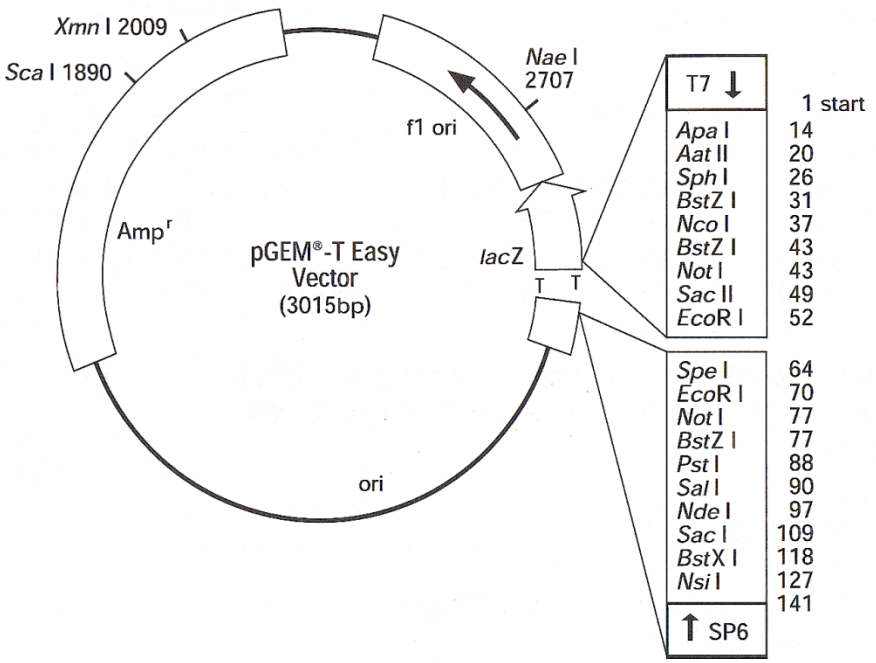
- 1. Isolation of DNA fragments from genomic DNA by selective amplification of a specific region of DNA
- 2. Generating probes in hybridization process: Southern, Western blot
- 3. Enabling analysis of DNA samples even from very small amounts of starting material

- 4. DNA sequencing
- 5. Screening
- 6. Forensic technique used to identify a person or organism by comparing experimental DNAs through different PCR-based methods

Cloning of PCR product

TA Cloning

- Doesn't use RE
- Easier and quicker than traditional subcloning
- Contained A and T at the end of sequences
- Eg PGEMT-Easy, pTOPO



Electrophoresis

- Techniques to separate and visually DNA fragments based on size
- Agarose (from seaweed, melted in buffer TAE, TBE depending on experimental)
- 0.5-2%(w/v)
- Visualization of migration of DNA by dye
- Migration proportional to DNA size
- Stained with ethidium bromide and observed under UV light

Visualization and dye

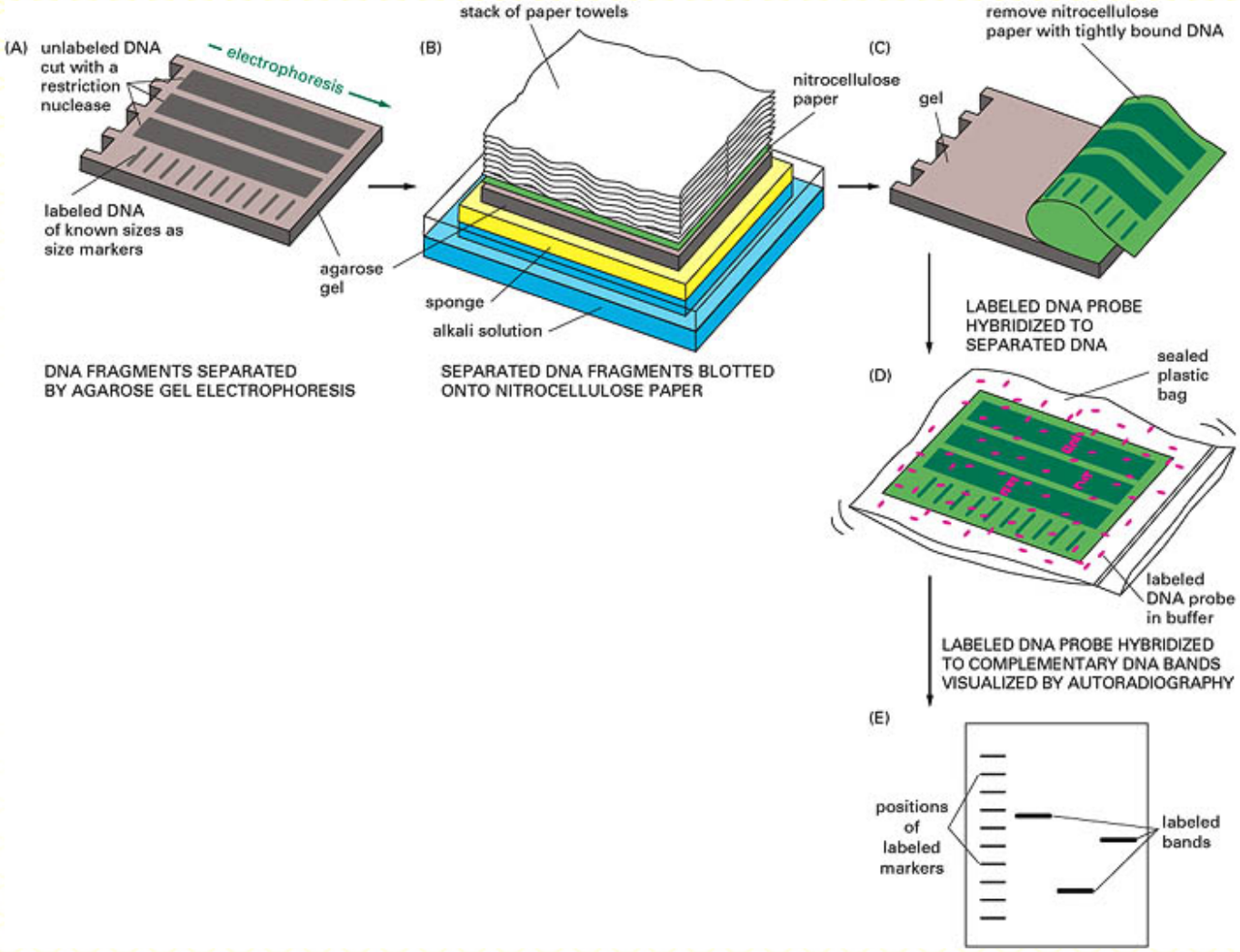
- Stain the gel with EtBr (Ethidium bromide)
- It fluoresces under UV light when intercalated into DNA (or RNA)
- Common loading buffers are xylene cyanol and bromophenol blue

DNA Sequencing

- Technique to determine the nucleotide sequence
- Knowing the DNA sequences of gene can be helpful:
  - To deduce the amino acids
  - To determine a protein encoded by a cloned gene
  - To identify regulatory elements such as promoter
  - To identify differences in genes created by gene splicing
  - To identify the genetic mutation

Southern Blotting

- To determine gene copy number
- Routinely used for detection of a specific DNA sequences
- The method is named after its inventor, Edwin Southern

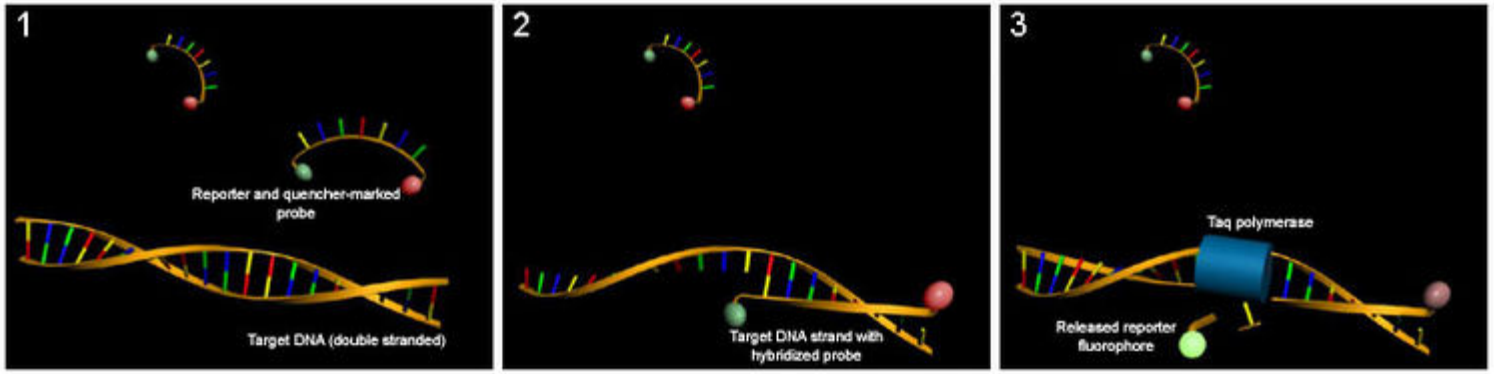

(A)
unlabeled DNA cut with a restriction nuclease
electrophoresis
labeled DNA of known sizes as size markers
agarose gel
DNA FRAGMENTS SEPARATED BY AGAROSE GEL ELECTROPHORESIS
(B)
stack of paper towels
nitrocellulose paper
sponge
alkali solution
SEPARATED DNA FRAGMENTS BLOTTED ONTO NITROCELLULOSE PAPER
(C)
remove nitrocellulose paper with tightly bound DNA
gel
LABELED DNA PROBE HYBRIDIZED TO SEPARATED DNA
(D)
sealed plastic bag
labeled DNA probe in buffer
LABELED DNA PROBE HYBRIDIZED TO COMPLEMENTARY DNA BANDS VISUALIZED BY AUTORADIOGRAPHY
(E)
positions of labeled markers
labeled bands

Gene Expression

- Involved gene expression study and regulation
- Mostly involved mRNA
- Involved:
  - Northern Blot; template was RNA
  - rtPCR:
    - allow detection of minute amount mRN
    - Convert RNA to cDNA by reverse transcription enzyme

Real Time PCR

- Others name: (Q-PCR/qPCR/qrt-PCR) or kinetic polymerase chain reaction (KPCR)
- To amplify and quantify targeted molecule
- Can be used to monitor PCR progress and amplified products
- Measure the DNA amplification
- Determine the presence and abundance of a particular DNA sequence in these samples

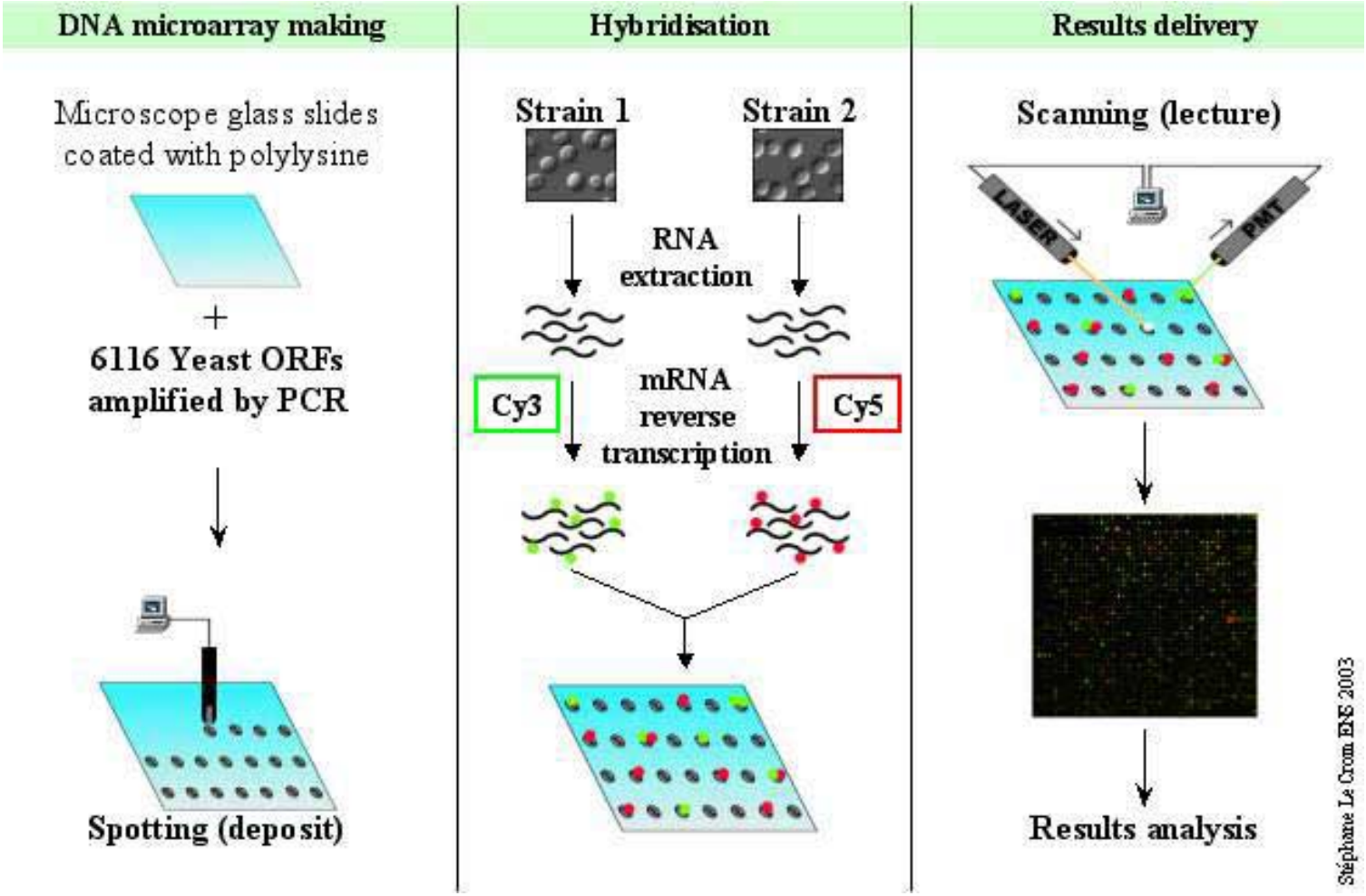


(1) In intact probes, reporter fluorescence is quenched. (2) Probes and the complementary DNA strand are hybridized and reporter fluorescence is still quenched. (3) During PCR, the probe is degraded by the Taq polymerase and the fluorescent reporter released.

Gene microarray

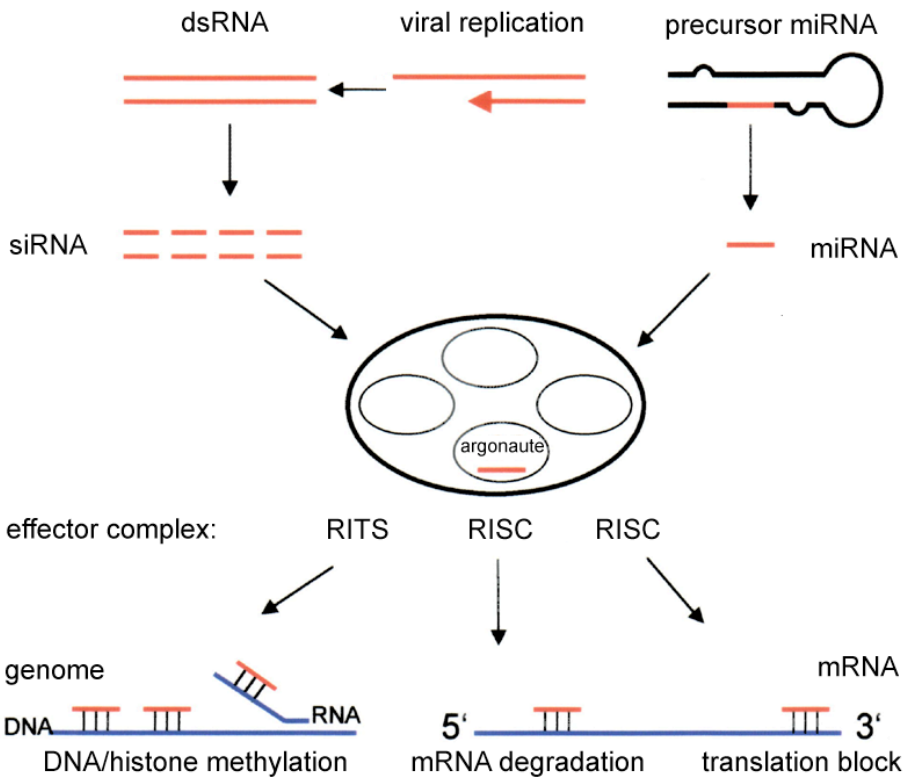
- Gene chip that contain complementary DNA sequence
- To test all genes expressed in the tissue
- It consists series of thousands of microscopic spots
- Specific DNA sequence; probes
- Hybridization of mRNA hybridize on the microarray

The making of DNA microarray



RNA interference (RNAi)

- Activation and its progress can be control
- Two types:
  - microRNA (miRNA)
  - Small interfering RNA (siRNA)
- Small RNAs can bind to specific other RNAs
- Increase or decrease their activity; prevention of mRNA to produce protein
- In eukaryotes
- Initiate by RNA-digesting enzyme; which cleaves long dsRNA into ~20nucleotides



The slicing enzymes produce siRNA from dsRNA and mature RNA (miRNA) from precursor miRNA. miRNA or siRNA is bound to an argonaute enzyme and an effector complex is formed, either RISC (RNA-induced silencing complex) or RITS (RNA-induced transcriptional silencing) complex.

Genomic and Bioinformatic

- Shotgun sequencing for small (4000 to 7000 bp)
- To apply the strategy, high-molecular-weight DNA is sheared into random fragments, size-selected (usually 2, 10, 50, and 150 kb), and clone into vector
- The entire genome, introns and exons is cloned and sequenced then individual genes are sorted out later through bioinformatic

Next generation sequencing

- Others technologies
- Production of shorter reads (25–500bp)
- Short time, cover high volume of data but expensive
- Least accurate

Bioinformatic & Genomic

- Interrelated fields that use of computer science to promote an understanding of biological processes
- Genomic: involved cloning, sequencing and analyzing whole genome
- Gene database : NCBI
- Accesssion number: Entry into gene bank
- Databases: BAC &YAC library, protein databases

- Activities in bioinformatics :
  - map and analyze protein and DNA sequences
  - Alignment of DNA and protein sequences
  - Compare between them
  - Creating and viewing 3-D models of protein structures

Human Genome Project

- An international project
- Determining the sequence of chemical base pairs
- To identify and map human genome
- Approximately 20,000–25,000 genes
- To understand the human genetic makeup
- Others organisms such as *E. coli*, the fruit fly, and the laboratory mouse

Medical Benefits

- Improved diagnosis of disease
- Earlier detection of predispositions to disease
- Rational drug design
- Gene therapy and control systems for drugs
- Pharmacogenomics “personal drugs”
- Organ replacement

DNA Forensics

- Identify potential suspects at crime scenes
- Exonerate wrongly accused persons
- Identify crime and catastrophe victims
- Establish paternity and other family relations
- Identify endangered and protected species as an aid to wildlife officials (prosecution of poachers)

Microbial Genome Research

- New energy sources (biofuels)
- Environmental monitoring to detect pollutants
- Protection from biological and chemical warfare
- Safe, efficient toxic waste cleanup

Agriculture and Livestock

- Disease-, insect-, and drought-resistant crops
- More nutritious produce
- Biopesticides
- Edible vaccines incorporated into food products
- New environmental cleanup uses for plants like tobacco