

## 7. Medical Applications of Molecular Biotechnology

Bi7430 Molecular Biotechnology

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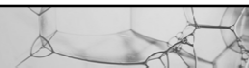
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### Outline



- Definition of red biotechnology
- Areas of red biotech applications
- Molecular diagnostics
  - immunological diagnostic methods
  - nucleic acid diagnostic systems
- Pharmacogenomics

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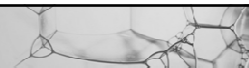
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### Red (medical) biotechnology



- biotechnology that deals specifically with **human health care** and methods of **treatment**
- aim at **prophylaxis**, accurate **diagnosis** and effective **treatment**
- personalised medicine** - therapy tailored based on patient profile rather than the "one size fits all" approach
- promising areas** of red biotech applications:
  - molecular diagnostics and genetic testing
  - vaccines, protein and nucleic acid therapeutics
  - tissue engineering and regenerative medicine
  - gene therapy and therapeutical cloning
  - drug delivery and nanomedicines

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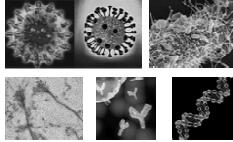
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## Clinical diagnostics

### □ success of modern medicine depends on **specific detection of**

- viruses
- bacteria
- fungi
- proteins
- nucleic acids



### □ medical laboratory methods contribute to **80% of diagnosis**

### □ good detection method should have three characteristics

- **sensitivity** - ability to detect small amounts of target molecule
- **specificity** - positive result for the target molecule only
- **simplicity** - ability to run efficiently, inexpensively on a routine basis

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## Clinical diagnostics

### □ **classical methods**

- cultivation, microscopic analysis, biochemical assays
- **POSITIVES**: simple, direct detection
- **NEGATIVES**: slow, laborious, low sensitivity, high skill level requirement, dangerous during cultivation infectious organisms

### □ **molecular diagnostics** (past 20 years)

- **immunological** and **nucleic acid diagnostic** systems
- **POSITIVES**: **fast, simple, high sensitivity, automatable, safe**
- **NEGATIVES**: not always specific, possible false positive or negative results

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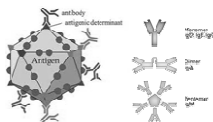
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## Molecular diagnostics

### **IMMUNOLOGICAL METHODS**

- sensitive, specific and simple
- based on **antigen-antibody interactions**
- **protein** >> sugar > nucleic acid
- wide range of applications:
  - **analysis of hormones, vitamins, metabolites, diagnostic markers** (e.g., insulin, testosterone, vitamin B12, prostaglandins, glucocorticoids)
  - **drug monitoring** (e.g., barbiturates, morphine, digoxin)
  - **detecting infection** (e.g., Legionella, HIV, hepatitis A, B)
  - **monitor cancer** (e.g., alpha-fetoprotein, carcino-embryonic antigen)



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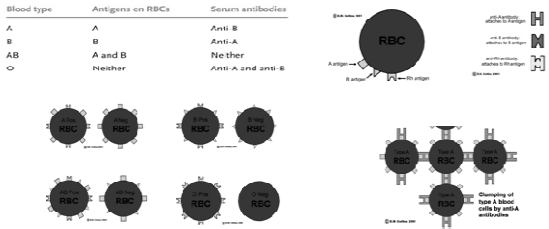
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## Molecular diagnostics

### IMMUNOLOGICAL METHODS

#### agglutination

- blood typing test in blood transfusion (ABO blood-group antigens have differences in the sugars on glyco-proteins)




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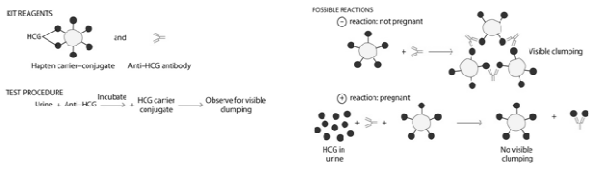
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## Molecular diagnostics

### IMMUNOLOGICAL METHODS

#### agglutination

- blood typing test in blood transfusion (ABO blood-group antigens have differences in the sugars on glyco-proteins)
- pregnancy kit (agglutination inhibition in presence of human chorionic gonadotropin, HCG, a glycoprotein hormone produced in pregnancy)




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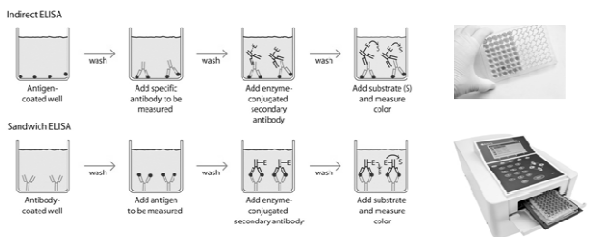
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## Molecular diagnostics

### IMMUNOLOGICAL METHODS

#### enzyme-linked immunosorbent assay (ELISA)

- enzyme based detection (e.g., HRP,  $\beta$ -galactosidase, alkaline phosphatase)
- fluorescence or colorimetric based detection




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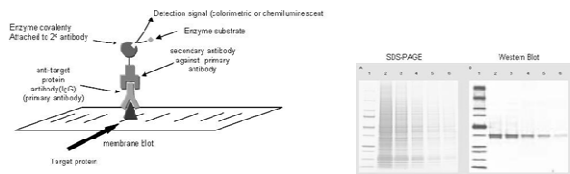
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## Molecular diagnostics

### IMMUNOLOGICAL METHODS

#### western blotting

- o **SDS-Page** - separates the components according to their molecular weight
- o **Blot:** the proteins in the gel are transferred to the sheet of nitrocellulose or nylon
- o **Immunoreaction:** after blocking probed with primary and secondary antibody
- o **Detection:** radioactive or fluorescence labeling, chemiluminescent, colorimetric




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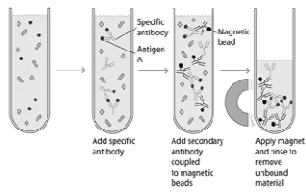
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## Molecular diagnostics

### IMMUNOLOGICAL METHODS

#### immunoprecipitation

immunoprecipitates collected by magnetic beads coupled to a secondary antibody




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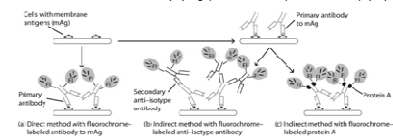
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## Molecular diagnostics

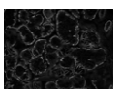
### IMMUNOLOGICAL METHODS

#### immunofluorescence

- o fluorescence labeled antibody (e.g., fluorescein, rhodamine, phycoerythrin)



- o fluorescence microscopy



lacrimal gland myoepithel



vaccinia virus infected cells



Chlamydomonas

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## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

- most common object for testing is **DNA**, in some cases **RNA**
- areas of medical applications:
  - **prenatal diagnostics**: non-invasive detection of fetal diseases (e.g., Down syndrome, cystic fibrosis)
  - **genetic testing**: high throughput testing for genetic disorders (e.g., SNPs markers, insertions, deletions)
  - **infectious diseases**: pathogen identification and detection of drug resistance (e.g. HIV, HBV, HCV)
  - **oncology**: early diagnosis of cancer (e.g., circulating tumor DNA, ratinoblastoma gene)
  - **transplantation medicine**: non-invasive, early detection of organ rejection (e.g., urine testing for kidney rejection, HLA typing)
  - **pharmacogenomics**: influence of genetic variation on drug response
- **DNA typing**: fingerprint of genotypic traits (paternity, crime suspects, ancestry)

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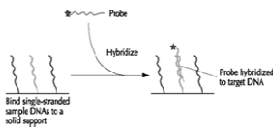
## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

- **DNA hybridization**
  - **probe** which anneals to the target nucleic acid
  - **bacterial and viral pathogens** contain specific gene(s)
  - **genetic diseases** caused by mutation or absence of particular gene(s)

#### CONVENTIONAL METHOD

1. attach the target DNA to a solid matrix
2. denaturation of both the probe and target
3. add the denatured probe, annealing to target
4. washing and detection (e.g. autoradiography, chemoluminescence, fluorescence)



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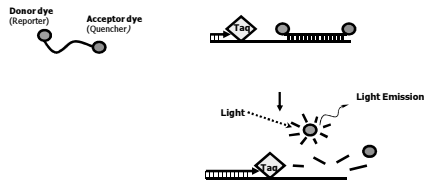
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## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

- **DNA hybridization**
  - **conventional method**
  - **TaqMan Probes** - hydrolysis by Taq polymerase



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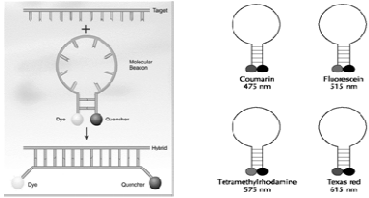
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## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

#### • DNA hybridization

- conventional method
- **TaqMan Probes** - hydrolysis by Taq polymerase
- **molecular beacons** - hairpin DNA with internally quenched fluorophore




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## Molecular diagnostics

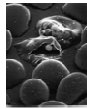
### NUCLEIC ACID DIAGNOSTIC SYSTEMS

#### • DNA hybridization

- conventional method
- **TaqMan Probes** - hydrolysis by Taq polymerase
- **molecular beacons** - hairpin DNA with internally quenched fluorophore

EXAMPLE: detection of parasite *Plasmodium falciparum*

- microscopic observations of blood smears is labour intensive
- **ELISA** does not differentiate between past and present infection
- **DNA diagnostic system** measure only current infection



Other examples: *Salmonella typhi* (food poisoning), *E. coli* (gastroenteritis)

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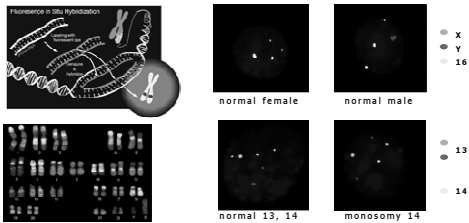
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## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

#### • DNA hybridization

- **fluorescence in situ hybridisation (FISH)**
  - new technique for **karyotyping**
  - chromosome **abnormalities** (segmental deletions and translocations)
  - **aneuploidy** (abnormal number of chromosomes)




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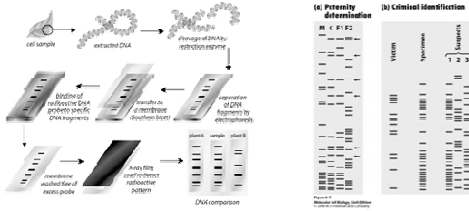
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## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

#### • DNA hybridization

- **DNA fingerprinting (DNA Typing)**
  - inherited **minisatellite DNA**, 10-30 repeats of 9-40 bp
  - identification of species, paternity, suspects and victims of crime/disasters
  - same DNA fingerprint one in  $10^7 - 10^8$




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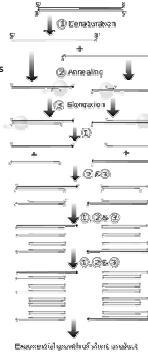
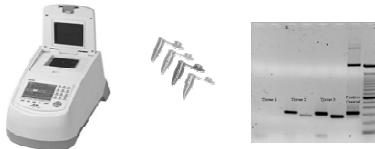
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## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

#### • polymerase chain reaction (PCR)

- **amplify single or few copies of DNA** to millions of copies
- the presence of the appropriate amplified size fragment confirms the **presence of the target**
- specific primers are available for the **detection of bacteria** (*E. coli*, *M. tuberculosis*), **viruses** (HIV), **fungi**
- early diagnosis of **malignant diseases** (leukemia)




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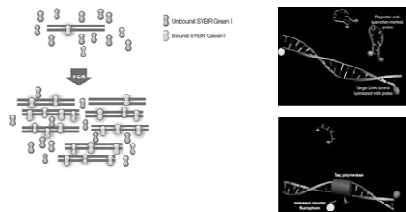
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## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

#### • polymerase chain reaction (PCR)

- **real-time PCR (Qpcr)**
  1. non-specific fluorescent dyes that intercalate with dsDNA
  2. sequence-specific DNA probes, oligonucleotides labeled with fluorescent reporter




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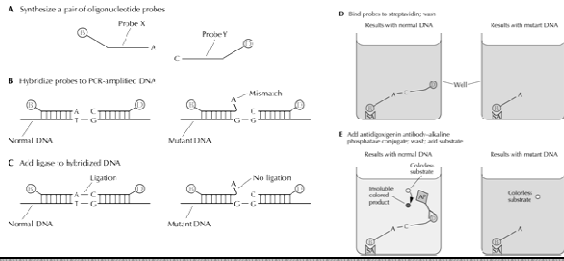




## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

- polymerase chain reaction (PCR)
  - oligonucleotide ligation assay (PCR/OLA)




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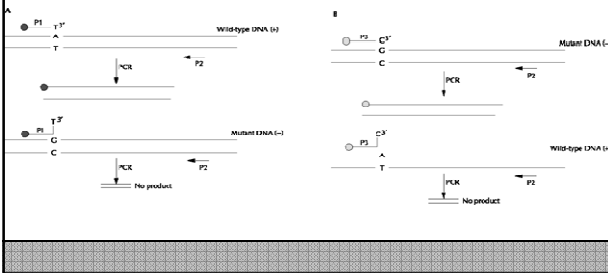
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## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

- polymerase chain reaction (PCR)
  - PCR using fluorescence-labeled primers
    - primer ends at SNP locus
    - mismatch gives no PCR product




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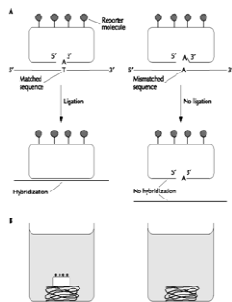
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## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

- polymerase chain reaction (PCR)
  - Padlock probes
    - probe complementary at ends (3 and 5)
    - ligate only if perfect match
    - only ligated forms attach to target




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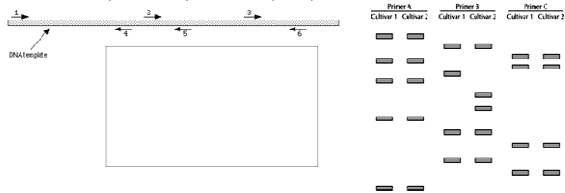
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## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

#### polymerase chain reaction (PCR)

- random amplified polymorphic DNA (RAPD)
  - DNA fingerprinting
  - „random“ primers used to produce DNA fingerprint
  - primers anneal in many places on template DNA and produce variety of sizes of amplified products




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## Molecular diagnostics

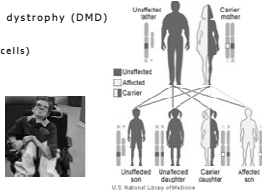
### NUCLEIC ACID DIAGNOSTIC SYSTEMS

#### DNA sequencing

- most direct method
- become cheap and fast, pushes other methods backward
- genes, genetic regions (i.e. gene clusters or operons), full chromosomes or entire genomes

EXAMPLE: Diagnostic for Duchenne muscular dystrophy (DMD)

- mutated dystrophin ("implosion" of muscle cells)
- X-linked recessive, carrier mother
- dystrophin gene large (2,4 Mb)
- first mutation carrier often mosaic (blood may not a mutation carrier)




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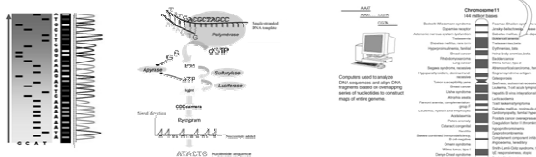
## Molecular diagnostics

### NUCLEIC ACID DIAGNOSTIC SYSTEMS

#### DNA sequencing

##### METHODS:

- Sanger sequencing
- pyrosequencing
- next generation methods (e.g., 454, SMART)
- 500 bp, primer walking 40 kbp




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# Pharmacogenomics

## CUSTOMIZED MEDICINE

- designing the most effective drug therapy and treatment strategies based on the specific **genetic profile of a patient**
- individuals react differently to the same drugs effects of genetic polymorphisms

Individuals respond differently to the anti-leukemia drug 6-mercaptopurine

The diversity in responses is due to variations (mutations,  $\Delta C/T$ ) in the gene for an enzyme called TPMT, or thiopurine methyltransferase.

After a simple blood test, individuals can be given doses of medication that are tailored to their genetic profile.

Most people metabolize the drug quickly. Doses need to be high enough to treat leukemia and prevent relapses.

Others metabolize the drug slowly and need lower doses to avoid toxic side effects of the drug.

A small portion of people metabolize the drug so poorly that its effects can be fatal.

Normal dose

Dose for an extra slow metabolizer (TPMT deficient)

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