LOSCHMIDT LABORATORIES

7. Medical Applications of Molecular Biotechnology

Outline

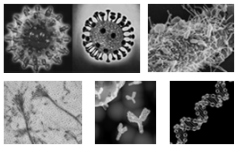
- Definition of red biotechnology
- Areas of red biotech applications
- Molecular diagnostics
  - immunological diagnostic methods
  - nucleic acid diagnostic systems
- Pharmacogenomics

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

Clinical diagnostics

- success of modern medicine depends on **specific detection of**
  - viruses
  - bacteria
  - fungi
  - proteins
  - nucleic acids



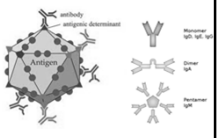
- medical laboratory methods contribute to **80% of diagnosis**
- good detection method should have three characteristics
  - **sensitivity** - ability to detect small amounts of target molecule
  - **specificity** - positive result for the target molecule only
  - **simplicity** - ability to run efficiently, inexpensively on a routine basis

Clinical diagnostics

- **classical methods**
  - cultivation, microscopic analysis, biochemical assays
  - POSITIVES: simple, direct detection
  - NEGATIVES: slow, laborius, low sensitivity, high skill level requirement, dengerous 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 possitive or negative results

Molecular diagnostics

IMMUNOLOGICAL METHODS



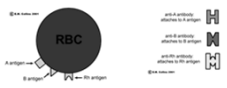
- sensitive, specific and simple
- based on **antigen-antibody interactions**
- **protein** >> sugar > nucleic acid
- wide range of applications:
  - **analysis of hormones, vitamins, metabolites, diagnostic markers** (e.g., insulin, testosterone, vitamin B12, prostaglandins, glucocorticoids)
  - **drug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

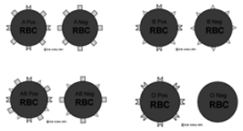
Molecular diagnostics

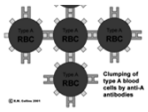
IMMUNOLOGICAL METHODS

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

Blood type	Antigens on RBCs	Serum antibodies
A	A	Anti-B
B	B	Anti-A
AB	A and B	Neither
O	Neither	Anti-A and anti-B



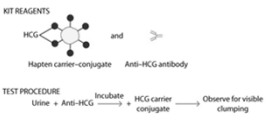


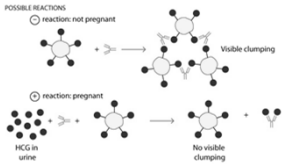


Molecular diagnostics

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)

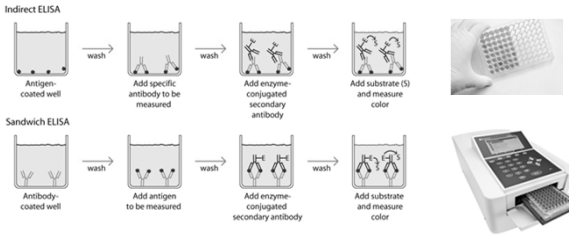




Molecular diagnostics

IMMUNOLOGICAL METHODS

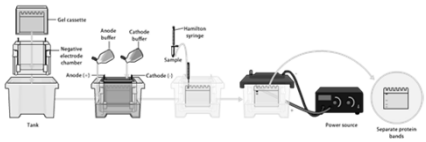
- **enzyme-linked immunosorbent assay** (ELISA)
  - enzyme based detection (e.g., HRP, β-galactosidase, alkaline phosphatase)
  - florescence or colorimetric based detection



Molecular diagnostics

IMMUNOLOGICAL METHODS

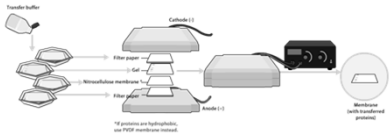
- **western blotting**
  - **SDS-Page** - separates the components according to their molecular weight



Molecular diagnostics

IMMUNOLOGICAL METHODS

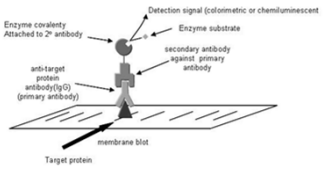
- **western blotting**
  - **SDS-Page** - separates the components according to their molecular weight
  - **Blot**: the proteins in the gel are transferred to the sheet of nitrocellulose or nylon

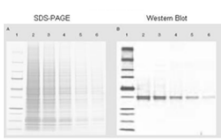


Molecular diagnostics

IMMUNOLOGICAL METHODS

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

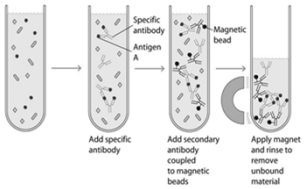




Molecular diagnostics

IMMUNOLOGICAL METHODS

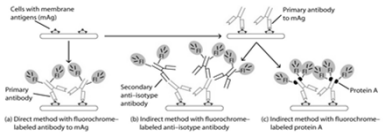
- **immunoprecipitation**
  immunoprecipitates collected by magnetic beads coupled to a secondary antibody



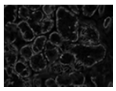
Molecular diagnostics

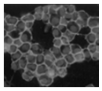
IMMUNOLOGICAL METHODS

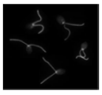
- **immunofluorescence**
  - fluorescence labeled antibody (e.g., fluorescein, rhodamine, phycoerythrin)



  - fluorescence microscopy


lacrimal gland myoepithel


virus infected cells


Chlamydomonas

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, human leukocyte antigen typing)
  - **pharmacogenomics**: influence of genetic variation on drug response
  - ***DNA typing:*** *fingerprint of genotypic traits (paternity, crime suspects, ancestry)*

Molecular diagnostics

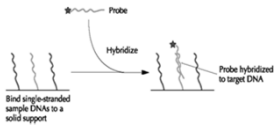
NUCLEIC ACID DIAGNOSTIC SYSTEMS

- **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)

Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

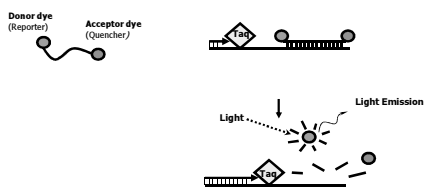
- **DNA hybridization**
  - **conventional method**
    1. attach the target DNA to a solid matrix
    2. denaturation of both the probe and target
    3. add the denatured probe, annealing to target
    4. washing and detection (e.g. autoradiography, chemoluminiscence, fluorescence)



Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

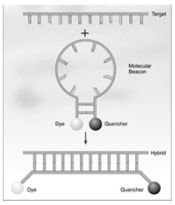
- **DNA hybridization**
  - **conventional method**
  - **TaqMan Probes** - hydrolysis by Taq polymerase

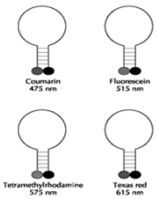


Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

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





Molecular diagnostics

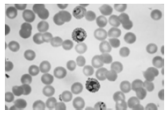
NUCLEIC ACID DIAGNOSTIC SYSTEMS

- **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

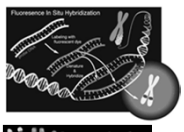
Other examples: *Salmonella typhi* (food poisoning)
Escherichia coli (gastroenteritis)

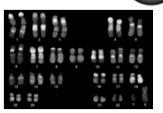


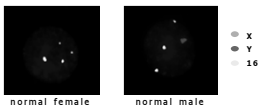
Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

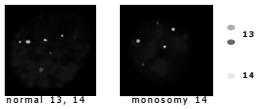
- **DNA hybridization**
  - **fluorescence *in situ* hybridisation (FISH)**
    - new technique for **karyotyping**
    - chromosome **abnormalities** (segmental deletions and translocations)
    - **aneuploidy** (abnormal number of chromosomes)







normal female normal male

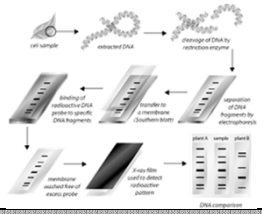


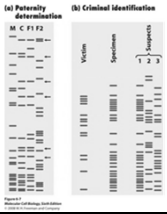
normal 13, 14 monosomy 14

Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

- **DNA hybridization**
  - **DNA fingerprinting (DNA Typing)**
    - inherited **minisatelite DNA**, 10-30 repeates of 9-40 bp
    - identification of species, paternity, suspects and victims of crime/disasters
    - same DNA fingerprint one in 10^5 - 10^8

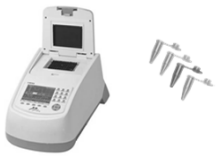


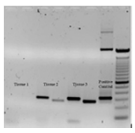


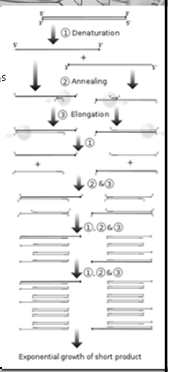
Molecular diagnostics

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)



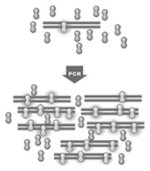


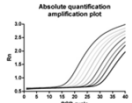


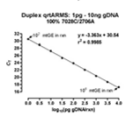
Molecular diagnostics

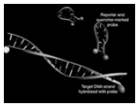
NUCLEIC ACID DIAGNOSTIC SYSTEMS

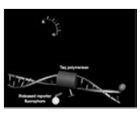
- **polymerase chain reaction (PCR)**
  - **real-time PCR (qPCR)**
    1. non-specific fluorescent dyes that intercalate with dsDNA
    2. sequence-specific DNA probes, oligonucleotides labeled with fluorescent reporter







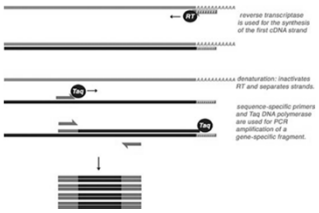




Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

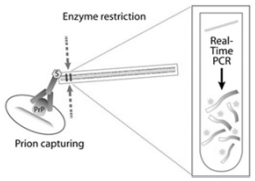
- **polymerase chain reaction (PCR)**
  - **reverse transcription PCR (RT-PCR)**
  - **real-time reverse-transcription PCR (qRT-PCR)**



Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

- **polymerase chain reaction (PCR)**
  - **immunoquantitative real-time PCR**
    - combines specificity of antibodies and sensitivity of PCR
    - overcome insufficient sensitivity of available immunological methods
    - sensitive for very low but still dengerous levels of pathogenic organisms



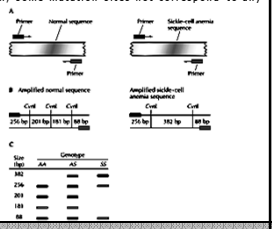
Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

- **polymerase chain reaction (PCR)**
  - **restriction fragment length polymorphism (RFLP)**
    - many diseases caused by single nucleotide change
    - method dependent on mutation within recognition site of restriction enzyme
    - even as many restriction enzymes known, some mutation sites not correspond to any

EXAMPLE: diagnostics of **sicle cell anemia**

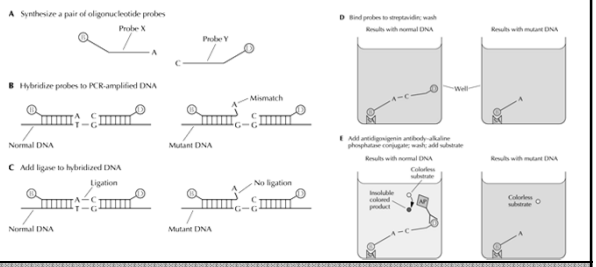
- anemia and damage to heart, lung, brain, joints and other organs
- single nucleotide change in the 6th aa of the beta-chain of hemoglobin (E6V)
- **normal DNA sequence CCTGAGG (A)**
- **mutant DNA sequence CCTGTGG (S)**
- homozygous state SS the red blood cells are irregularly shaped



Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

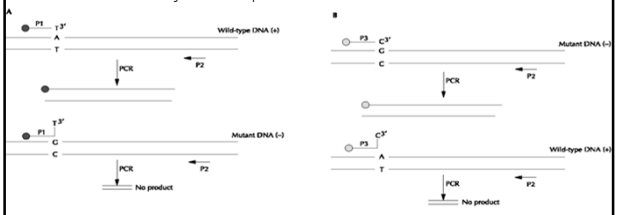
- **polymerase chain reaction (PCR)**
  - **oligonucleotide ligation assay (PCR/OLA)**



Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

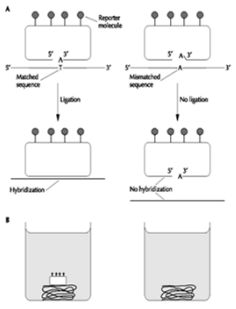
- **polymerase chain reaction (PCR)**
  - **PCR using florescence-labeled primers**
    - primer ends at SNP locus
    - mismatch gives no PCR product



Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

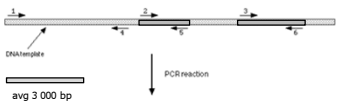
- **polymerase chain reaction (PCR)**
  - **Padlock probes**
    - probe complementary at ends (3´ and 5´)
    - ligate only if perfect match
    - only ligated forms attach to target

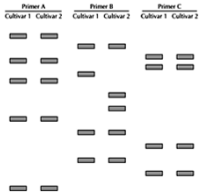


Molecular diagnostics

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





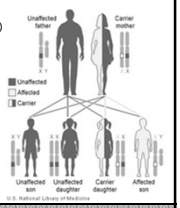
Molecular diagnostics

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



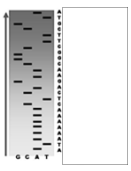
Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

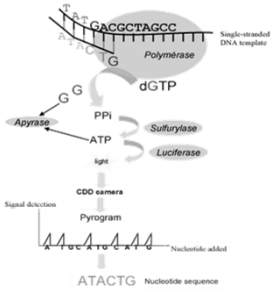
- **DNA sequencing**

METHODS:

- **Sanger sequencing**
- **pyrosequencing**
- **next generation methods** (e.g., 454, SMRT)



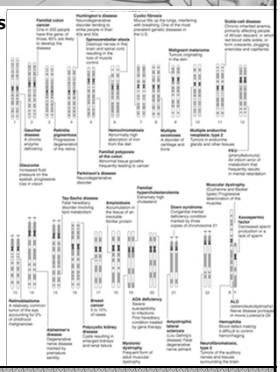
Nobel Price in 1958 and 1980



Molecular diagnostics

NUCLEIC ACID DIAGNOSTIC SYSTEMS

- **DNA sequencing**
- **genom sequencing**
  - **Human Genom Project**
    (10 years and 3 billion USD)
  - **Jamese Watson genom**
    (2008, several month)
  - **Archon X Prize**
    (100 genoms/10 days, 10 000 USD/genom)



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