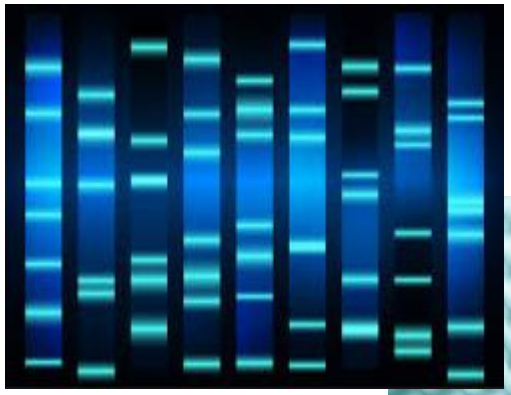
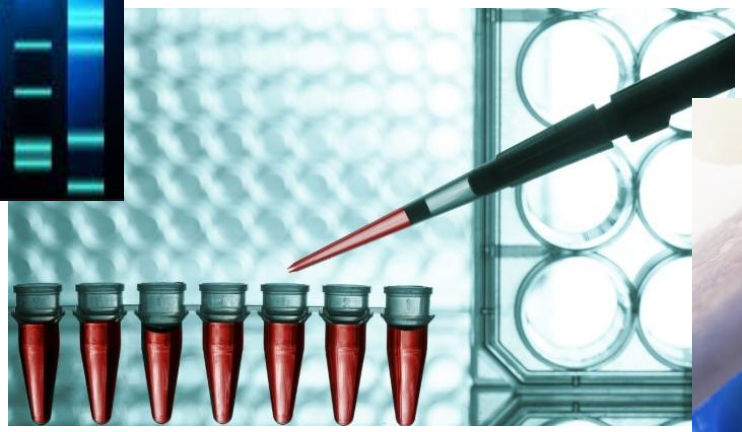
Molecular Diagnostics Laboratory

Summer Semester 2020





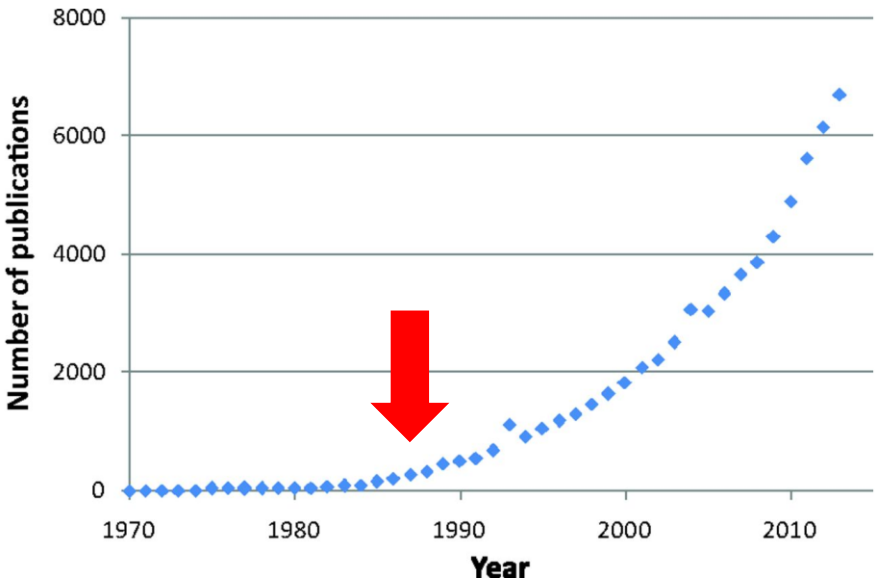
Why Molecular Diagnostics?

Collection of analytical tools used for the detection and/or analysis of biological markers either at the genetic or protein levels

Applications:

- **Oncology**
- Infectious diseases
- Genetic diseases
- Autoimmune diseases
- Forensics
- Drug screening
- Parental & prenatal screening

PubMed was searched with terms: “molecular diagnos*” (in the title or abstract)



Molecular diagnostic publications, 1970–2013

Outline

- Organizational issues
- Final report
- Protocols
- Schedule

Outline

- **Organizational issues**
- Final report
- Protocols
- Schedule

Organizational issues

- Where?

Bogner-Strauss Lab
Humboldstrasse 46/ III
ZMB - Zentrum für molekulare Biowissenschaften

Please be on time!!

- When?

Group 1:
03.03: 12.30-18 hr
04.03: ?-18 hr
05.03: 10.30-18 hr (lunch break)
06.03: 8-12 (Backup day)

Group 2:
09.03: 12.30-18 hr
10.03: ?-18 hr
11.03: 10.30-18 hr (lunch break)
12.03: 8-12 (Backup day)

Organizational issues

- Groups

Leila

Melina

Johannes

Organizational issues

- Other important points
  - Social room available
  - You can bring own laptop (waiting times)
  - Lab coats 
  - Mobile phones 
  - Gloves 
  - Lab protocol (read and printed!) 
  - Kitchen available
  - Many eating options for lunch break!

Outline

- Organizational issues
- **Final report**
- Protocols
- Schedule

Final report

- Deadline: ?
- Individual – Groups?
- Parts:
  - Abstract
  - Introduction
  - Materials and methods
  - Results
  - Discussion

Final report

- **Abstract: What it says?**
  - The report in a paragraph
  - Past tense
- **Introduction: Why I did it?**
  - Tell the reader what the report is about => Decision!
  - Present tense
  - Aprox. 1 page

Final report

- **Materials and methods: What I did?**
  - Include technical specifications, sources and amount of materials used => Protocol
  - Past tense
- **Results: What I found?**
  - Include a clear and concise outline of the experiments you did and the results yielded
  - Graphs, tables, etc.
  - Headings mirror those of M&M
  - Past tense

Final report

- **Discussion: What it means?**
  - What the results show, whether there are any exceptions
  - The relationship of your results to previous work
  - Conclusions you reached and why you reached them
  - Past tense for own findings
  - Present tense for other peoples work
  - Aprox.1 page

Final report

- **Additional considerations**
  - Units
  - Abbreviations
  - No table of contents needed
  - Pictures can be added
  - References

Outline

- Organizational issues
- Final report
- **Protocols**
- Schedule

Protocols

❖ Measurment of blood sugar levels

How does a glucometer works?

- ❖ Electrochemical method: Redox reaction
- ❖ Test strips contains **glucose oxidase**
- ❖ **Step 1**: Glucose oxidation by enzyme

$$\text{glucose} + \text{GOD}_{(ox)} \rightarrow \text{gluconolactone} + \text{GOD}_{(red)}$$

$$\text{glucose} \rightarrow \text{gluconolactone} + 2e^-$$

$$2e^- + \text{GOD}_{(ox)} \rightarrow \text{GOD}_{(red)}$$

BLOOD GLUCOSE CHART

Mg/DL	Fasting	After Eating	2-3 hours After Eating
Normal	80-100	170-200	120-140
Impaired Glucose	101-125	190-230	140-160
Diabetic	126+	220-300	200 plus

- ❖ **Step 2**: Enzyme reoxidization by mediator

$$\text{GOD}_{(red)} + 2\text{Fecp}_2\text{R}^+ \rightarrow \text{GOD}_{(ox)} + 2\text{Fecp}_2\text{R} + 2\text{H}^+$$

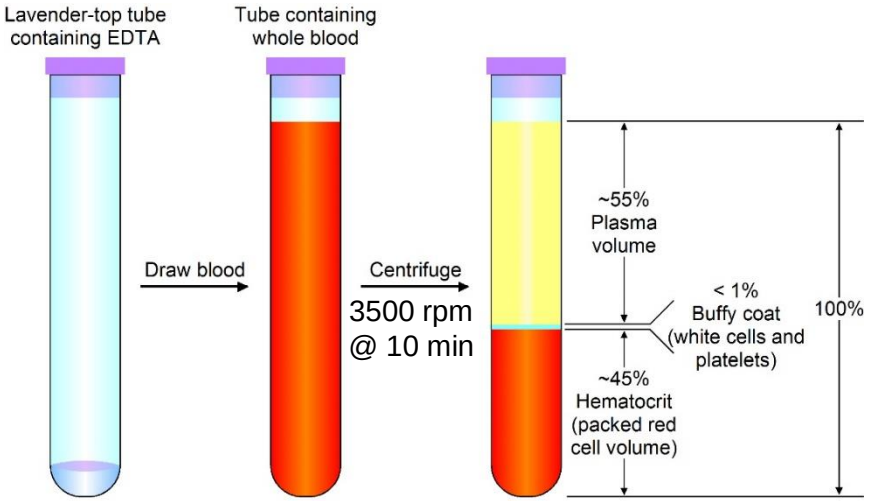
$$2\text{Fecp}_2\text{R} \rightleftharpoons 2\text{Fecp}_2\text{R} + 2e^-$$

Volunteers?

Protocols

❖ Triglycerides (TGs) measurement from blood plasma I/III

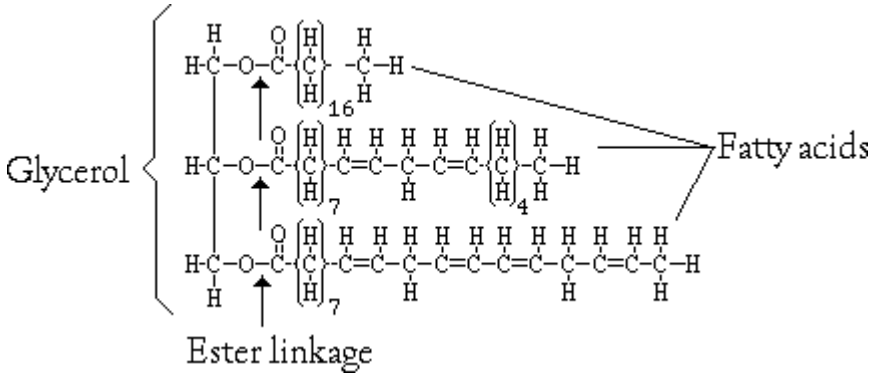
- ❖ Cholesterol, **TGs**, and high-density lipoproteins are important constituents of the lipid fraction of the human body
- ❖ Plasma is the non-cellular fraction of blood
- ❖ It is mostly water (up to 95% by volume), and contains dissolved salts, proteins, **lipids**, sugars, clotting factors, hormones, CO_2 and O_2



Protocols

❖ Triglycerides (TGs) measurement from blood plasma II/III

❖ Clinical significance



Triglycerides levels and risk for heart disease

Meaning	Level in mg/dL
Normal Range: Low risk	< 150
Borderline High	150 - 199
High	200 - 499
Very high: Highest risk	> 500

Measurement of TGs is important in the **diagnosis and management of hyperlipidaemias**. Eg. Diabetes mellitus, atherosclerotic disease, endocrine disorders

Protocols

❖ Triglycerides (TGs) measurement from blood plasma III/III

❖ Assay methodology

1. $\text{Triglycerides} + H_2O \xrightarrow{Lipase} \text{Glycerol} + \text{Free Fatty acids}$

TGs are enzymatically hydrolysed by lipase to free fatty acids and glycerol

2. $\text{Glycerol} + \text{ATP} \xrightarrow{GK} \text{Glycerol-3-phosphate} + \text{ADP}$

The glycerol is phosphorylated by ATP with glycerol kinase (GK) => glycerol-3-phosphate and ADP

3. $\text{Glycerol-3-phosphate} + O_2 \xrightarrow{GPO} \text{DAP} + 2H_2O_2$

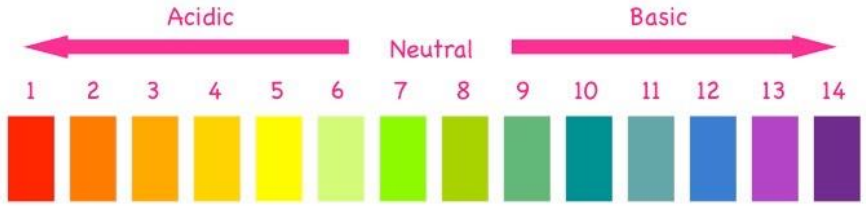
Glycerol-3-phosphate is oxidised by dihydroxyacetone phosphate (DAP) by glycerolphosphate oxidase => H_2O_2

4. $H_2O_2 + \text{4-AAP} + \text{3,5 DHBS} \xrightarrow{POD} \text{Quinoneimine dye} + 2H_2O$

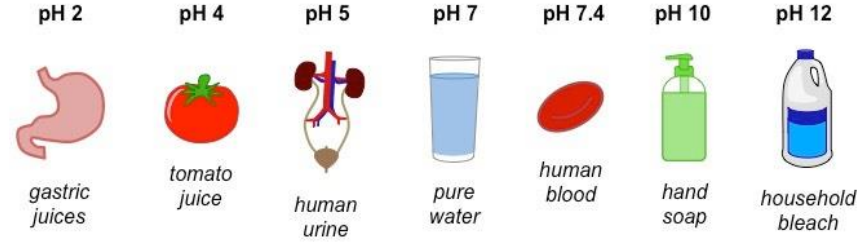
The H_2O_2 reacts with 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzene sulfonate (DHBS) to produce a red coloured dye. The absorbance of this dye is proportional to the concentration of TGs present in the sample

Protocols

❖ pH measurements



Examples of pH Conditions:

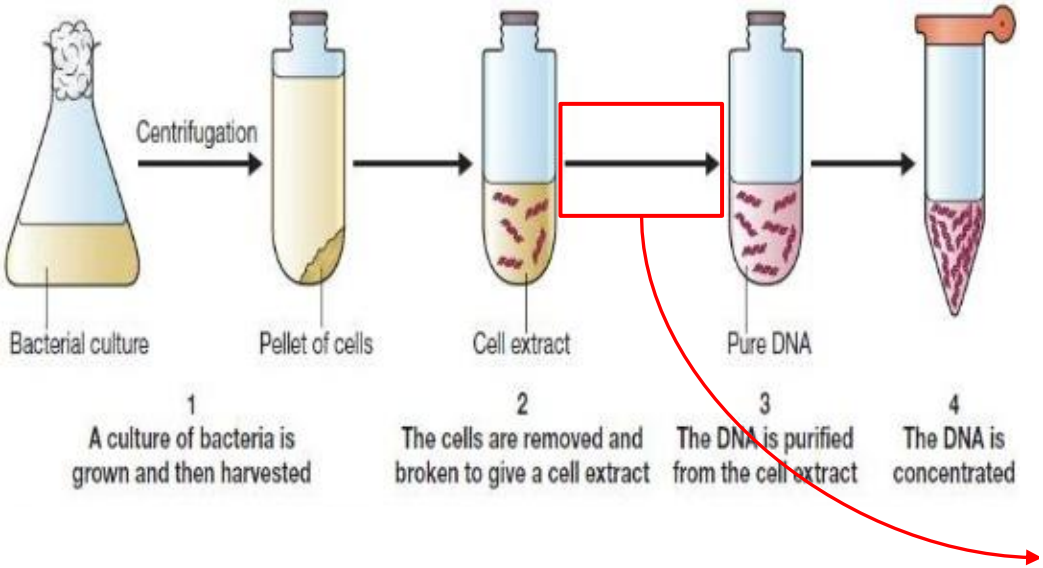


pH
(acidity/alkalinity of the urine)

- The normal values range from 4.6 to 8.0.
- A high urine pH (alkaline urine) may indicate:
  - Gastric suction
  - Renal failure
  - Renal tubular acidosis
  - Urinary tract infection
  - Vomiting
- A low urine pH (acidic urine) may indicate:
  - Chronic obstructive pulmonary disease
  - Diabetic ketoacidosis
  - Diarrhea
  - Starvation
- The drugs acetazolamide, potassium citrate, or sodium bicarbonate can increase pH; Ammonium chloride, chlorothiazide diuretics, and methenamine mandelate can decrease urine pH

Protocols

❖ Plasmidic DNA isolation from bacterial cells



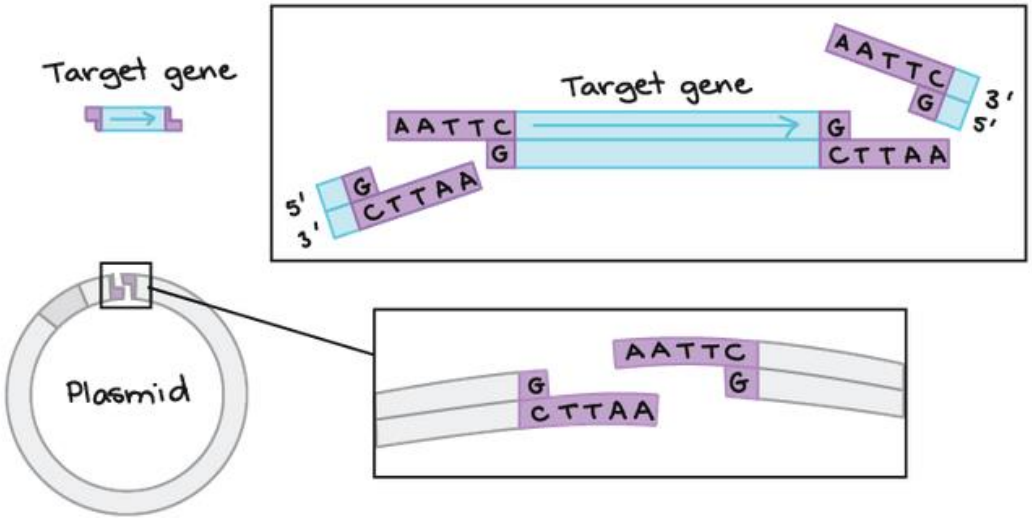
6. Plasmidic DNA isolation from bacterial cells

Execution:

- Pipette 1,5ml of a bacterial overnight culture into a reaction tube and centrifuge it at 5000 rpm for 5 minutes
- Discard or remove the supernatant via pipetting (carefully, so that you don't remove the pellet)
- Resuspend the pellet in 50µl of the P1 buffer by pipetting it up and down
- Add 300µl of the TENS buffer and mix it via inverting the tube five times
- Add 100µl of the 3 M sodium acetate solution and mix it again
- Centrifuge the mix at 5000 rpm for 10 minutes
- Pipette the supernatant to a new reaction tube and discard the pellet
- Add 1ml of ice cold 100% ethanol to the supernatant and mix it
- Incubate the mix at -20°C overnight
- Centrifuge the mix at 12000 rpm for 10 minutes
- Remove the supernatant carefully und wash the pellet with 0,5 ml 70% ethanol by inverting the tube carefully
- Remove all of the ethanol and let the pellet dry for 10 minutes (open the tube)
- Resuspend the pellet in 30 µl distilled water by pipetting it up and down
- The solution contains your isolated plasmid DNA (sample A)

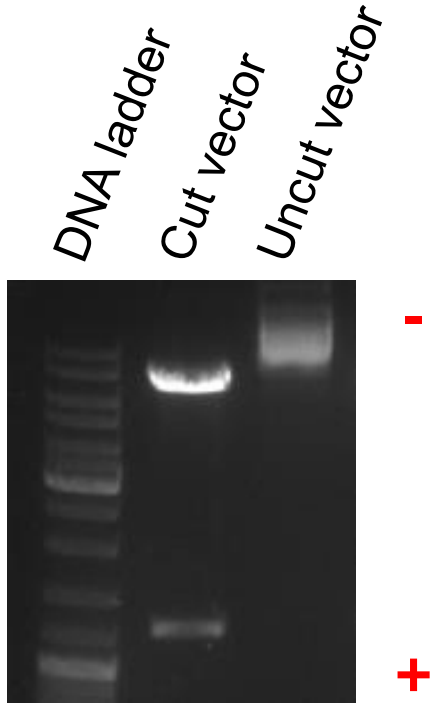
Protocols

Enzyme cutting of plasmidic DNA



https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/overview-dna-cloning

DNA gel electrophoresis

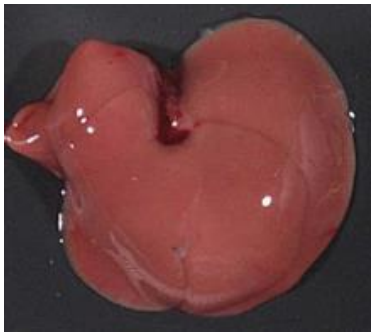


Protocols

- **Western blot**
  - Commonly used to separate proteins and then identify a specific protein of interest
  - Steps (2 days):
    - Protein isolation from mouse liver
    - Bicinchoninic acid assay (BCA): To determine total protein concentration
    - Gel electrophoresis (polyacrylamide)
    - Transfer to membrane (nitrocelullose)
    - Blocking
    - Antibodies incubation
    - Detection

Protocols

❖ Protein isolation for Western blot

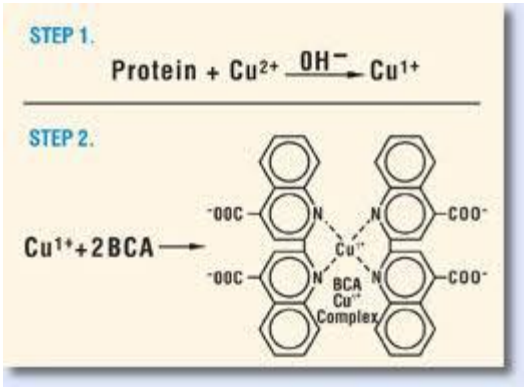


For protein isolation:

1. Weight approximately 60 mg of tissue and mix it with 2 ml of RIPA buffer + PIC (1:2000) in a homogenizer tube with 15 ceramic beads.
2. Homogenize it 2x20 seconds @ 6500 rpm
3. Leave it on ice for 5 min
4. Centrifuge 15 minutes, 10000 rpm @ 4°C
5. Transfer the upper phase into a new 1.5 ml tube
6. Centrifuge 30 minutes, 13000 rpm @ 4°C
7. Transfer the upper phase into a new 1.5 ml tube
8. Samples are now ready to proceed with the protein estimation

Protocols

❖ Protein quantification for Western blot: Bicinchoninic acid assay (BCA):

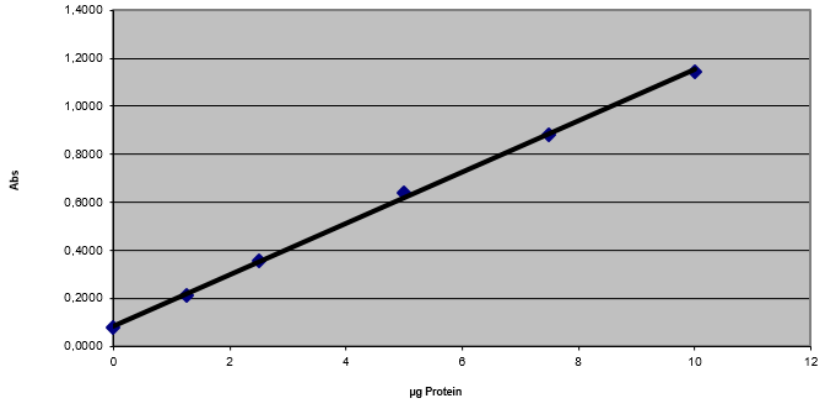


The assay consists in two reactions:

1. The peptide bonds in protein reduce Cu^{2+} ions to Cu^{+} (The amount of Cu^{2+} reduced is proportional to the amount of protein present in the solution)

2. Two molecules of BCA chelate with each Cu^{+}, forming a purple-colored complex that strongly absorbs light at a wavelength of 562 nm.

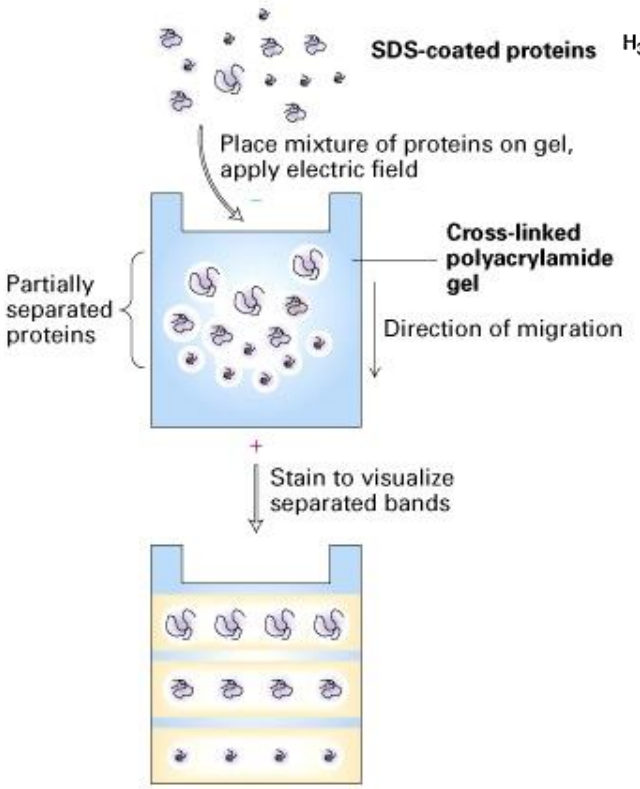
Samples to be used: murine liver

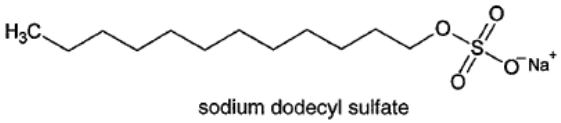


Protocols

❖ Western blot

1. SDS-polyacrylamide gel electrophoresis (PAGE):



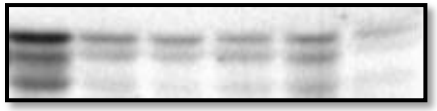


sodium dodecyl sulfate

2. After SDS-PAGE:

- ❖ Transfer to a membrane
- ❖ Ponceau staining

- ❖ Blocking
- ❖ Antibodies incubation (1ry and 2ry)
- ❖ Detection



Outline

- Organizational issues
- Final report
- Protocols and background
- **Schedule**

Schedule

Task / Day	03/10.03	04/11.03	05/12.03	06/13.03
Introduction to pipetting				
Protein isolation & BCA				
Blood sugar measurement				
TG assay blood plasma				
pH measurements				
Western Blot				
Bacterial DNA isolation				
DNA enzyme cutting				
DNA gel electrophoresis				

Please let us know in advance if you want to visit the animal facility!

Thank you for your attention!!