Pharmaceutical Biology Practice book

University of Pécs, Faculty of Pharmacy
Pharmaceutical Biology

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Laboratory work, accident and fire safety rules

In order to protect our fellow students and our own safety in the laboratory, all students are obliged to observe the following rules.

Laboratory rules:

1) Students may enter the laboratory only with the permission of the teacher, and only the supervising teacher, assistant teacher or students participating in the practical training are allowed in the room during the training.

2) Students are only allowed to bring into the laboratory the equipment necessary for their work, e.g. writing utensils, minutes. Other equipment (bag, jacket) can be stored in the place provided by the Department during the internship.

3) **Wearing the lab coat is compulsory for all students** and must be taken care of individually. Protective gloves and goggles will be provided by the Department of Education. During the internship, it is recommended to keep long hair together

4) It is **forbidden** to eat, drink, chew or taste any substances in the laboratory.

5) During work, order and cleanliness must be maintained, spilled liquids and chemicals must be wiped up immediately.

6) No individual experimentation is allowed, only work under the instructions of the teacher!

7) The improper use of laboratory equipment such as fire extinguishers, etc. is **forbidden.**

8) In the laboratory, it is important to use the equipment properly. Only use equipment that is necessary for the exercise.

9) It is the student's responsibility to tidy up the workbenches and keep the equipment clean at the end of the exercise.

General rules on health and safety at work and accident prevention:

1) Do not work with broken or cracked glassware!

2) Do not wash the cut with water, unless corrosive or toxic substances have been involved. Carefully apply pressure, allow the wound to bleed and make sure that no glass fragments remain, then disinfect the wound area. Apply a plaster to a small wound and sterile gauze to a large wound.

3) Use protective gloves when using flammable, corrosive or toxic substances.
4) Only take from a labelled bottle and store chemicals in such a container. Do not replace the lids of chemical bottles or jars, always place them on the table with the lid on. This way you do not contaminate the chemical or the table. Wipe up any spills or splashes immediately.

5) Solid chemicals should be removed only with a clean chemical spoon, which should be washed after use.

6) Do not pour any remaining solvent back into the chemical bottle from which it was removed. It must be stored in a designated collection container in the laboratory. Do not pour organic solvent down the drain!

General fire safety rules:

1) The National Fire Safety Code defines five classes of fire hazard, designated by the letters "A" "B" "C" "D" "E".

   "A" - Highly flammable and explosive

   "B" - Danger of fire and explosion

   "C" - Danger of fire

   "D"- Moderately flammable

   "E"- Not liable to catch fire

   Always be aware of the flammability class of the material you are working with.

2) When extinguishing a fire, one of the following factors must be removed or eliminated:

   -combustible material
   -combustible medium (oxygen)
   -ignition temperature

3) Extinguishing a fire should always be started as soon as possible, taking into account the properties of the burning material and the size of the fire, and by a rapid and comprehensive survey of the available suitable equipment and materials.

4) An attempt should be made to extinguish the fire with the equipment and tools available.

5) In the event of a fire in or near electrical installations, the power must be disconnected before extinguishing is started.

6) There are different ways to put out a fire:
   - for a small fire, e.g. a table fire: in case of solvent ignition (a few ml), cover the fire with a damp cloth, blanket, cooking pot, cut off oxygen, possibly extinguish with water.

- In case of a personal fire: extinguish by lying down quickly on the ground with burning clothing in a rolling motion. If necessary, use a safety shower!
- in case of a laboratory fire:

A. Use of a fire extinguisher: a device from which extinguishing agent can be directed into the fire by the pressure in the device when it is put into operation. The most suitable devices for extinguishing small, incipient fires.

Different types of extinguishers are suitable for extinguishing different types of fire. Based on the properties of combustible materials, the different classes of fire are as follows:

Fire class A: Fire of solid organic materials

Fire class B: Fire involving solid liquids or liquids in solid form

Class C: Flammable gases

Fire class D: Metal fires

Division F: Fires involving oils and fats

Types of fire extinguishers:

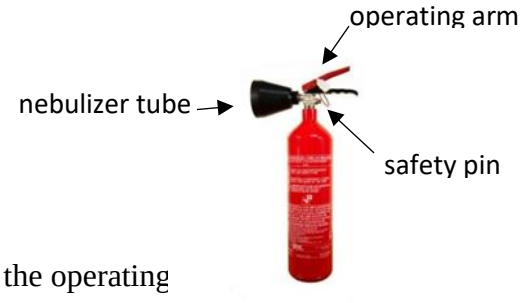
I. Powder extinguishing: The extinguisher has CO_2 in the tank, the pressure is used to extinguish $NaHCO_3$ powder. The $NaHCO_3$ decomposes in the fire and the evolving CO_2 blocks the fire from oxygen. It can be used to extinguish class A, B and C fires. Not suitable for extinguishing electrical appliances!

II. Foam extinguisher: Generally suitable for extinguishing class A and B fires, but there are extinguishers with extinguishing capacity which can also be used for class F fires. Foam extinguishing is prohibited in the presence of electricity, as foam is an extinguishing agent mixed with water.

III. Gas extinguishing: extinguishing with carbon dioxide. It is generally used to extinguish fires of fire class B or electrical fires, as it does not damage the equipment. Carbon dioxide evaporates on contact with the burning material, does not attack it, does not conduct electricity and is therefore preferably used for the transport and storage of liquids, valuable materials and objects, foodstuffs and flammable and explosive materials.

IV. Water extinguishing: Water is generally used as an extinguishing agent for the combustion of solid materials. Water extinguishing is prohibited in the presence of electricity, and water extinguishing is also prohibited for solid chemical substances that may react with water.

Extinguishing a burning person with a fire extinguisher is **FORBIDDEN!**



Operating a carbon dioxide extinguisher:

1. removing the fuse pin

2. directing the fog hose to the edge of the fire

3. apply extinguishing agent by intermittent pressure on the operating

The low temperature of the extinguishing agent may cause a risk of frostbite and therefore requires extra care.

B. Wall-mounted hydrants are water intakes for extinguishing fires and are located in a cabinet with fittings and accessories in buildings. Their location shall be indicated by the words "hydrant". Wall hydrants are not only intended to support the work of firefighters, but also, and above all, to facilitate rapid intervention and fire-fighting by building occupants and workers. They may only be used after the power has been switched off! Use must be considered in the event of water damage.

7) Immediately cool burned skin with plenty of running water, then treat with a burn ointment or spray. In severe cases, seek medical attention.

8) In the event of electric shock, first disconnect the main switch in the laboratory. In case of loss of consciousness, take the casualty to fresh air, place him in a stable side position and call a doctor.

9) Before opening the main shut-off valve of the gas line, check that the valves of each burner are closed, then put them into operation. When the work is finished, make sure that the gas burner tap on the tables and the main shut-off tap are closed.

10) Do not lean over the gas burner while working.

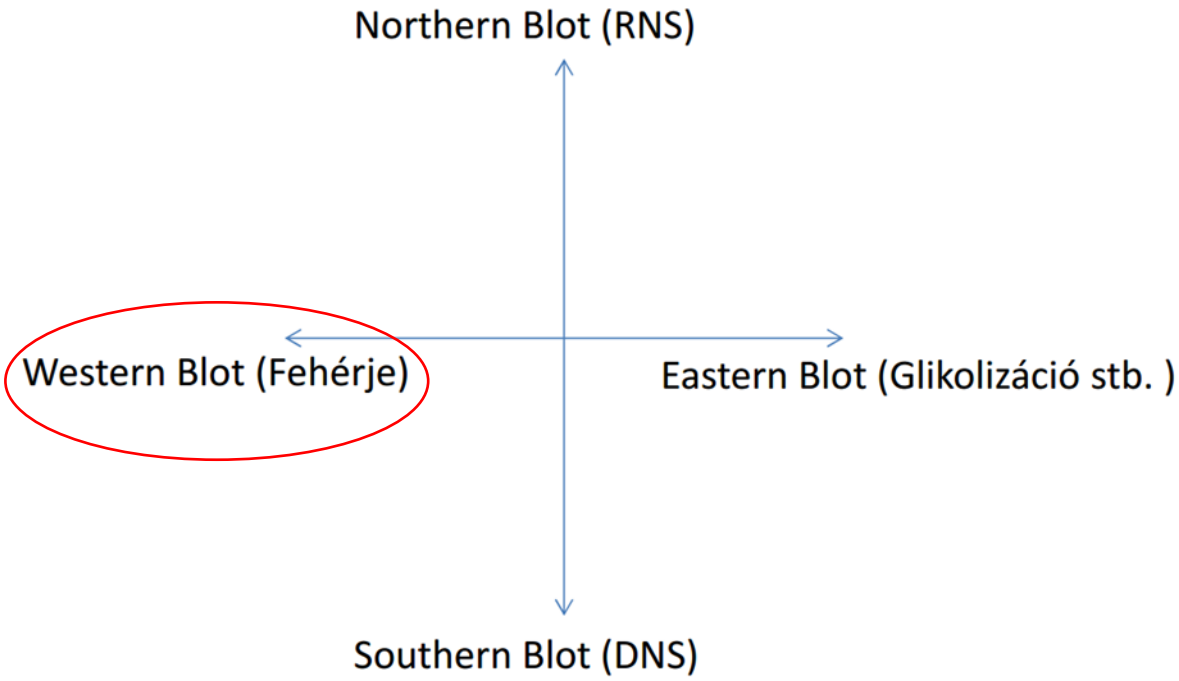
11) To prevent accidents, extinguish or light the Bunsen burners when not in use.

12) Ensure that all gas and water taps are turned off and electrical equipment is switched off before leaving the laboratory after work is completed.

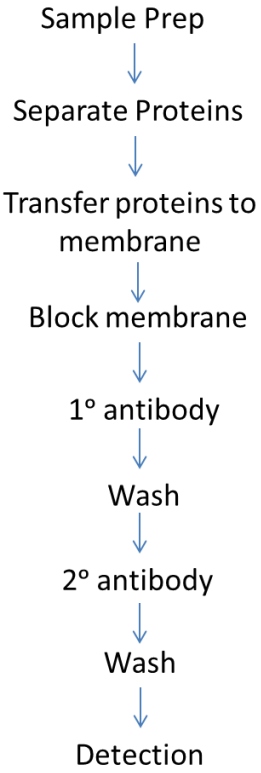
In case of sickness, injury or any accident, contact the teacher immediately.

1. Western Blott

A way of detecting a specific protein in a sample.



Western Blott's steps:



I.Sample Prep and Quantification

- Lysis depends on tissue!
  - Culture Cells ⟶ sonicate
  - Tissue samples ⟶ homogenise
- Centrifuge to remove debris
- Keep cold and use protease inhibitors and phosphatase inhibitors!
- If you want to compare samples, you need to load the same amount of protein in each sample.
- Accurate quantification important
- Denaturation
- Bradford assay
- BCA and Lowry assays. Less variable, but more sensitive to pH, detergents, EDTA etc in lysis buffer
- A280. Accurate, but sensitive to DNA contamination.

II. Separate Proteins

1) SDS-PAGE

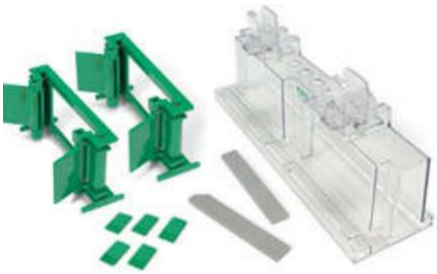
In order to see proteins, we need to separate them out.

- SDS-PAGE -> Separation of proteins in sample according to molecular weight/mass
  - Proteins are denatured before SDS-PAGE.
  - Pick gel, buffer, running conditions appropriate for size of protein.
- 2D-PAGE.

2) GEL PRODUCTION

10%	Separating
Acrylamide	3 ml
Dest.water	3.6 ml
Separating buffer (1.5M TRIS)	2.25 ml
10% SDS	90 ul
10% APS	75 ul
TEMED	30 ul
10%	Stacking (upper, with pockets)
Acrylamide	0.53 ml
Dest.water	2.45 ml
Stacking buffer (0.5M TRIS)	1 ml
10% SDS	40 ul
10% APS	50 ul
TEMED	10 ul

After added the catalyst TEMED - Tetramethylethylenediamine and the radical initiator ammonium persulfate (APS) the polymerisation is started.



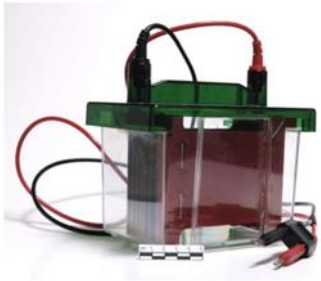
I. Transfer proteins to membrane

Transfer separated proteins onto a membrane, which can then be probed with antibodies to detect the protein of interest.

Membrane can be Nitrocellulose or PVDF

Types of transfer:

Wet



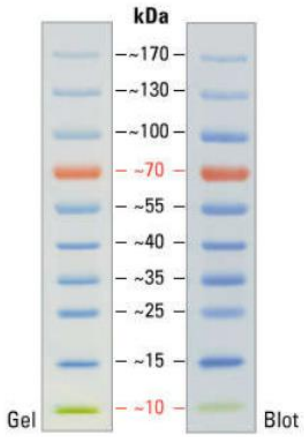
Best for proteins >100kDa

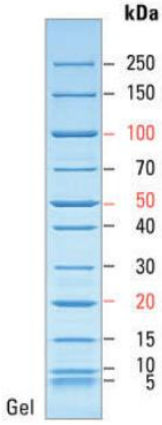
Semi-dry

Quick

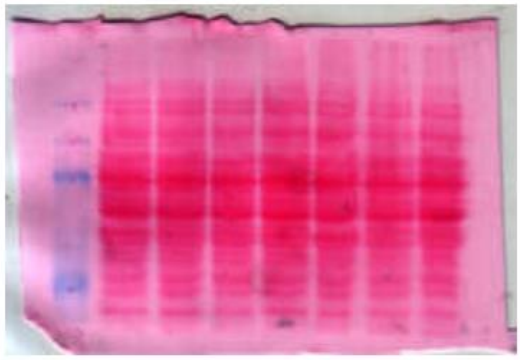
Dry

Even quicker





Ponceau staining



II.Blocking

Fill up the space on the membrane to prevent non-specific antibody binding.
Recommended to block for >1 hour.

Milk	***BSA***
Strong blocking agent Less signal Not-recommended for phospho-proteins Cheap!	High signal High background!

Diluted in same Buffer use for washing (PBST/TBST)

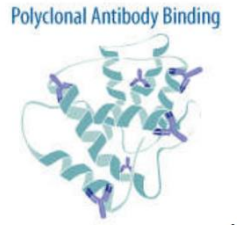
III. Primary Antibodies

Antibody specific to your protein of interest.

- Monoclonal
- Polyclonal

Monoclonal:one antigen sequence – highly specific, low background, but low signal

- Commercial monoclonal ~£200 for 100□l
- Custom monoclonal >£2000



Polyclonal: multiple antigen sequences – more background, high signal

- Custom monoclonal £600-800

- Directly conjugated HRP – fewer steps, lower signal
- Amount of antibody –determined empirically (1/1000 -1/5000)
- Incubate in wash buffer, or blocking buffer for 1-2 hours at RT or overnight at 4°C

IV. Washing

Aim: Wash off unbound antibody.

- Tris-Buffered Saline or Phosphate-Buffered Saline
- Tween-20 or Triton-X-100 (or other detergents beginning with T)
- In most cases, it doesn't matter which you use … but … for phospho-proteins TBST may be better.

V.Secondary Antibody

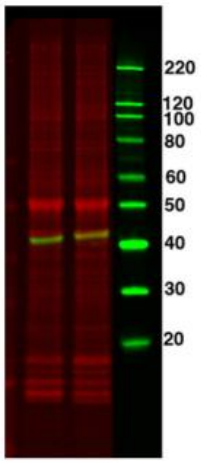
Antibody against IgG of primary antibody.

- Conjugated to a reporter – HRP, Alexa 488 etc.
- Dilution in range of 1/10000 – 1/100000 in blocking buffer
- Incubate for ~2 hours at RT.

VI.Washing

VII. Detection

- Colorimetric – less sensitive
- Radioactive label
- Fluorescently labelled secondary antibody – highly quantitative
- Chemiluminescent – HRP or AP labelled secondary antibody - very sensitive!



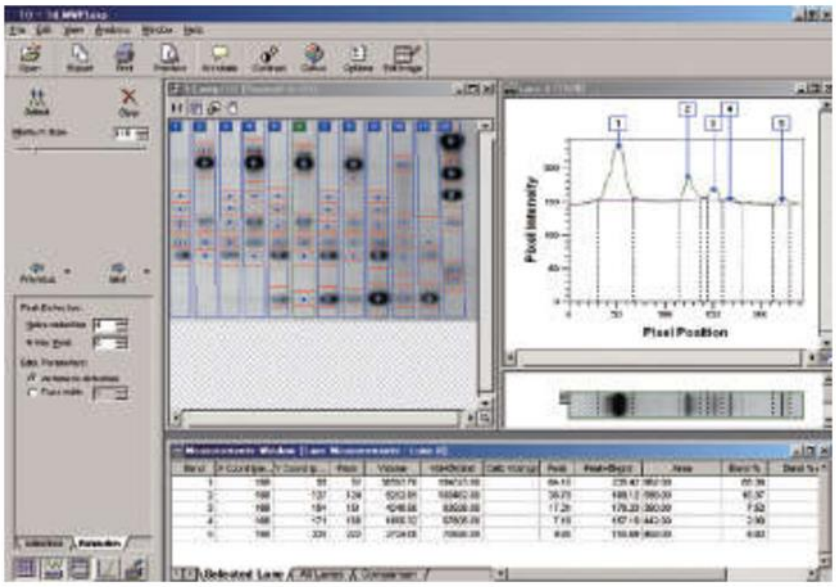
<u>Chemiluminescent detection:</u>

- Mix 2 solutions together → pipette onto membrane
- Wait 5 minutes

Quantification

- ImageQuant software allows quantification of blot results.

- Bear in mind, signal is not linear!



Questions:

1. What problems can arise when using this method?

2. List the advantages of Western Blott?

3. What are the disadvantages of the Western Blott method?

4. Why is a blocking step necessary?

2. RNA isolation with Trizol reagent

Caution! Trizol reagent and the chloroform are corrosive and harmful! Toxic in contact with skin and if swallowed. Causes burns. After contact with skin, wash immediately with plenty of detergent and water. Wear protective lab coat and gloves! Wearing safety googles is highly recommended! RNases can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper technique.

TRIZOL Reagent (U.S.Patent No.5,346,994) is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent, a mono-phasic solution of phenol and guanidine isothiocyanate.

Protocol summarised:

1. **Homogenisation.**
- Add 0.5 ml Trizol to 50 – 100 mg tissue then homogenise.

2. **Phase separation.**
- Incubate the sample for 3' @ RT (room temperature) to permit the complete dissociation of nucleoprotein complexes.
- Add 0.1 ml chloroform, close the tube and shake vigorously for 15".
- Incubate for 3' @ RT.
- Centrifuge for 10' @ 12 000 x g. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colourless upper aqueous phase. RNA remains exclusively in the aqueous phase.

3. **RNA precipitation.**
- Carefully pipette the upper phase into a new tube. Do no touch the interphase!
- Add 0.5 ml isopropyl-alcohol.
- Incubate for 3' @ RT
- Centrifuge for 7' @ 12 000 x g.
- The RNA precipitate forms a gel-like pellet on the side and bottom of the tube.

4. **RNA wash.**
- Remove supernatant by pipetting.
- Add 1 ml 75% ethanol to the pellet.
- Vortex then centrifuge for 5' @ 7 500 x g.

5. **Redissolving the RNA.**
- Carefully remove the ethanol by pipetting
- Let the pellet dry for 5 -10' @ RT.
- Redissolve the RNA in 50 µl of distilled water.

Further steps: quantitation of RNA concentration and purity. cDNA synthesis.

Questions:

1. Why is it necessary to wear gloves during the process?

2. What are the physiological roles of RNases?

3. What can contaminate the RNA sample?

4. What are the advantage of Trizol?

3. Plasmid DNA isolation from competent bacteria

Plasmids

Functions: Protection of DNA, transport of DNA into the cells, replication in the host cells

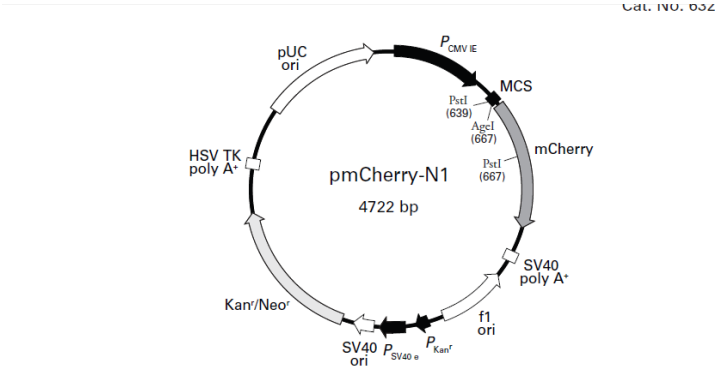
- Nonessential, extra chromosomal, circular, double stranded DNA molecules
- Ability to promote autonomous replication
- Plasmids are found in the nature

Natural plasmids:

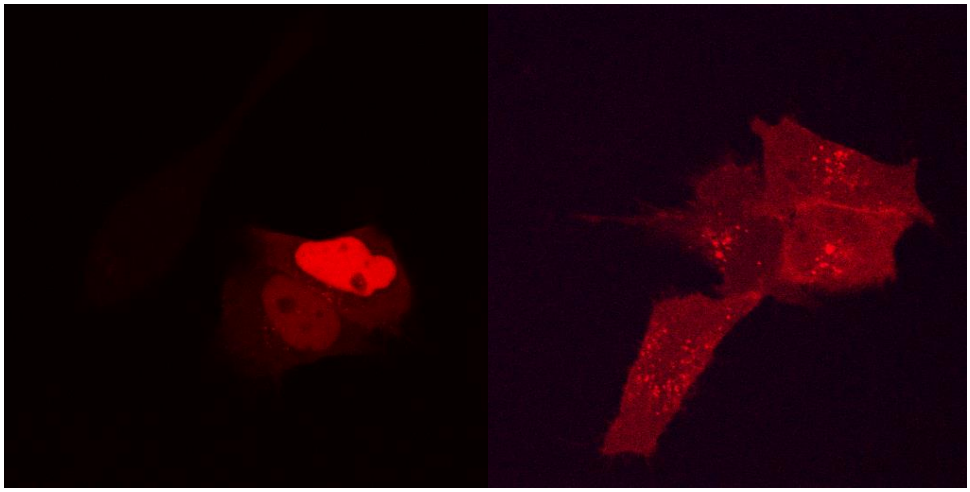
- Their size is 3-20 kb
- contain resistance genes which help in the survival and/or help in the adaptation to the environment
- Plasmids are able to move from one cell into another cell

Artificial plasmids:

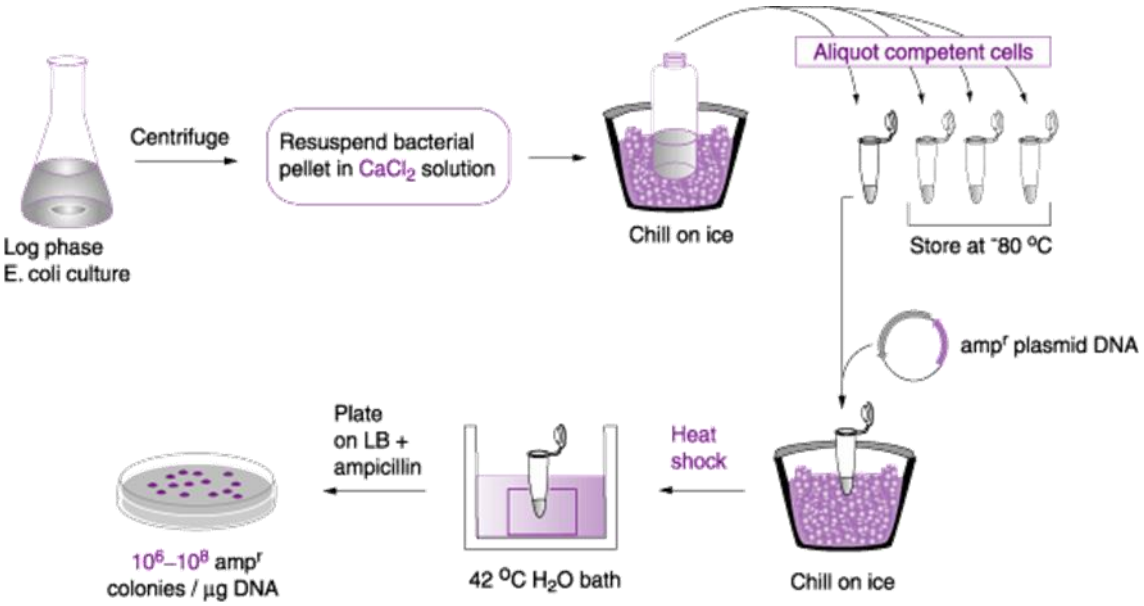
- They are smaller: 3-6 kb,
- modular structure
- multiple cloning site/polylinker region with unique restriction sites
- Contain a genetic marker (usually dominant) for selection
- Their 3D structures are protected against mechanical affects
- They are present in multiple copies (low copy or high copy plasmids)
- Two types: cloning and expression vectors
- cloning plasmids: get the target DNA sequence in the cells
  (However big DNA sequence cannot be inserted into the plasmid because it becomes instable could be broken and the insert could be cracked from the plasmid.)
- Expression plasmids: mRNA and then protein are transcribed and translated from the inserted DNA; the sequence of the promoter must have complementary structure with the mRNA polymerase of the target cell (weak and strong promoters)
- ATG and STOP codons
- Protein selection: tags e.g. RFP, GST, His, HA, Myc etc. Purification of the proteins with tag specific antibodies
- fluorescent tag: *in vivo* examination of the protein
- protein+RFP tag



protein+RFP tag



Transformation of competent bacteria



Buffers and solutions

Sol I.
Sol II.
Sol III.
Absolute ethanol
80% ethanol
sterile distilled water

Sol I.	Sol II.	Sol III.
25mM Tris-HCl pH 8 10mM EDTA pH 8 0,1mg/ml RNase A	0,2M NaOH 1% SDS	3M K- acetate pH 4.8 (5M K- acetate + cc. acetic acid)

Isolation of pTriex-3 neo plasmid from E. coli, DH5α strain

1. Centrifuge 1,5 ml of bacterial culture in a microcentrifuge tube for 1 min at full speed.
2. Discard the supernatant.
3. Add 150µl Sol I to the pellet, and mix it by vortexing for 2 x 30 sec.
4. Add 150µl Sol II to the suspension and rinse 6-8 times.
5. Add 200µl Sol III to the suspension and rinse 6-8 times.
6. Centrifuge the sample 10 min at full speed.
7. Pipette the supernatant into a new microcentrifuge tube.
8. Add 1 ml of absolute ethanol to the supernatant and rinse 5-6 times
9. Centrifuge the tube 5 min at full speed.
10. Discard the supernatant.
11. Add 1 ml 80% ethanol to the pellet and centrifuge 2 min at full speed.
12. Remove the supernatant carefully using a pipette.
13. Dry the pellet for 15 min at room temperature.
14. After the evaporation of ethanol resuspend the pellet in 50µl sterile distilled water.

Isolation of genomic DNA from E.coli cells

DiaExtract Microbial DNA Isolation Kit

This kit performs quick isolation of genomic DNA from a wide range of microbial samples. The system is designed for use either in traditional lysosyme-mediated lysis or in destruction-homogenisation instrumental lysis of bacteria, monocellular fungi and other microbes of diverse samples.

The use of homogenization device gives an optimized way to perfectly homogenize the samples by destructing their matrices. The mechanical destruction provides an extremely quick, efficient and well-reproduced homogenization in contrast to extraction methods with enzymatic degradation. Samples are placed into 2.0 ml destruction tubes containing rough glass particles and stainless steel balls. Homogenization in any destruction device takes place in the presence of Lysis Buffer. If the laboratory has no benchtop destruction device, the traditional way for lysing the samples is advised, supplementing the starting Lysis Buffer with lysosyme. Following lysis, samples are centrifuged to pellet the debris and by the help of Binding Matrix, double stranded DNA of the supernatant will be bound selectively. DNA is then purified and desalinated with Wash Buffer. Finally, the eluted DNA is ready for digestion, electrophoresis, PCR, or any other molecular applications.

Protocol

1. Pipet 1,5 ml bacterial culture into an eppendorf tube.

2. Centrifuge at 10000 rpm for 1 min

3. Discard the supernatant.

4. Repeat steps from step 1-3 with the same tube.

5. Add 1 ml Lysis buffer onto the pellet.

6. Resuspend the pellet by pipetting up and down.

7. Pipet the suspension into a destruction tube containing rough glass particles and stainless steel balls.

8. Vortex the tube for 2 x 1 min.

9. Centrifuge the sample at 10000 rpm for 1 min.

10. Pipet 600 µl of supernatant into a new eppendorf tube.

11. Add 200 µl Binding matrix to the sample.

12. Mix gently with shaking for 5 min.

13. Centrifuge the tube at 3000 rpm for 1 min.

14. Discard the supernatant.

15. Pipet 500 µl Wash buffer onto the matrix and mix them by pipetting up and down.

16. Centrifuge the tube at 3000 rpm for 1 min.

17. Discard the supernatant.

18. Repeat steps 15-17.

19. Pipet 50 µl PCR grad water onto the matrix, mix it well by gently shaking for 2 min.

20. Centrifuge the tube at 10000 rpm for 2 min.

21. Pipet the supernatant into a new eppendorf tube.

Determination of DNA concentration by spectrophotometer

1. Dilute 10 µl sample to 300 µl with sterile distilled water in a microcentrifuge tube.

2. Suspend the sample by pipetting up and down and pipet the sample into a quartz cuvette/plastic UV transparent cuvette.

3. Calibrate the photometer to distilled water (pipet 300 µl dist. water into the cuvette)

4. Measure the UV absorbtion of the sample (absorbance).

Determination occurs at 260 nm.

Formula of quantification:

OD x dilution factor x 50µg/ml (dsDNA) = µg DNA/ml

50= the optical density of the double stranded DNA of 50 µg/ml (concentration) is 1.

Determination of DNA concentration by Implen nanophotometer

1. Calibrate the photometer with 2 µl distilled water.

2. Pipet 2 µl sample onto the cuvette without dilution.

The photometer determines the absorbtion of the sample at 260, 280, 230 and 320 nm.

The 260/280 ratio shows the protein content of the sample. It is good if the value is above 1,8.

The absorption at 230 nm shows the ethanol carryover. 260/230>1.5

The absorption at 320 nm shows the particles in the sample.

3. The photometer determines the DNA concentration of the sample in ng/µl.

Questions:

1. Why is it important to subclase buffer solvents when isolating the pTriex-3 neo plasmid?

2. What is the purpose of DNA isolation?

3. Why is calibration necessary when using a spectrophotometer or nanophotometer?

4. Calculation: determination of DNA concentration:

OD:

Dilution factor:

4. Agarose gel electrophoresis

Choose the suitable agarose based on the size of the DNA molecule.

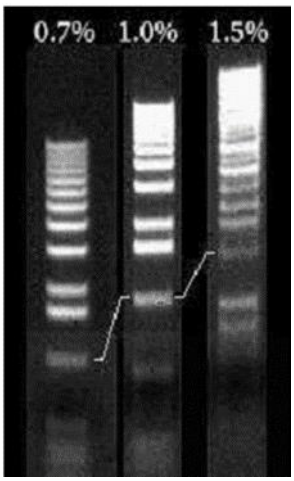
Running Buffers:

- TAE- Tris-acetate-EDTA pH 7,6-7,8
- TBE- Tris-borate-EDTA pH 8,3

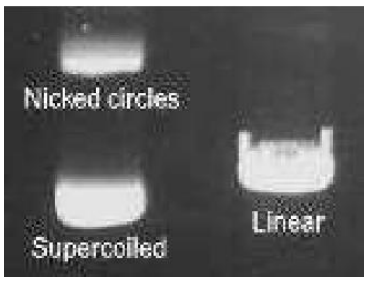
Use in appropriate dilution!

<u>Influencing factors of DNA running:</u>

1. Size of the DNA molecule: the speed of running is inversely proportional to the logarithm to the base ten of the molecular weight.
2. Concentration of agarose: the logarithm of the DNA electrophoretic mobility directly proportional to the gel concentration.



3. Conformation of the DNA: same sized closed circular, nicked circular and linear DNA molecules run with different speed.



4. Applied current: at low voltage the linear DNA molecule runs directly proportional to the voltage. At high voltage the relative large fragments run faster than the smaller ones (5V/cm).

Loading buffers

Role: the loading buffer holds the DNA at the bottom of the well, doesn't let the DNA diffuse out from the gel.

- Negatively charged dye
- Bromphenol-blue, Xylen-cyanol
- Glycerol or sucrose
- Factory-made loading dyes: contain different types of dyes in one loading buffer

Visualization:

- Ethidium bromide: intercalating dye, less sensitive, carcinogen, DNA harming effect; advantage: it can be mixed into the loading, into the gel or the gel can be dyed after running, Detection wavelength: UV
- SYBR dyes (green, gold…etc.): intercalating dye, high sensitivity; advantage: only small amount is enough, its carcinogen effect is not known, it can be mixed into the loading, into the gel, detection wavelength: visible light (blue light)
- Silver dye: very sensitive, expensive

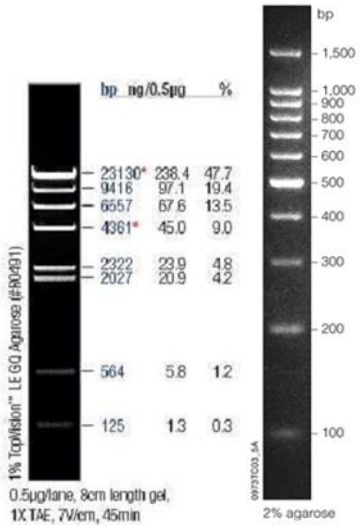
How much DNA is needed to be run on gel?

- Depends on the method of visualization
- Approx. 20 ng DNA is well seen
- If we use too much DNA, it is difficult to determine its size

Molecular weight markers/DNA ladders

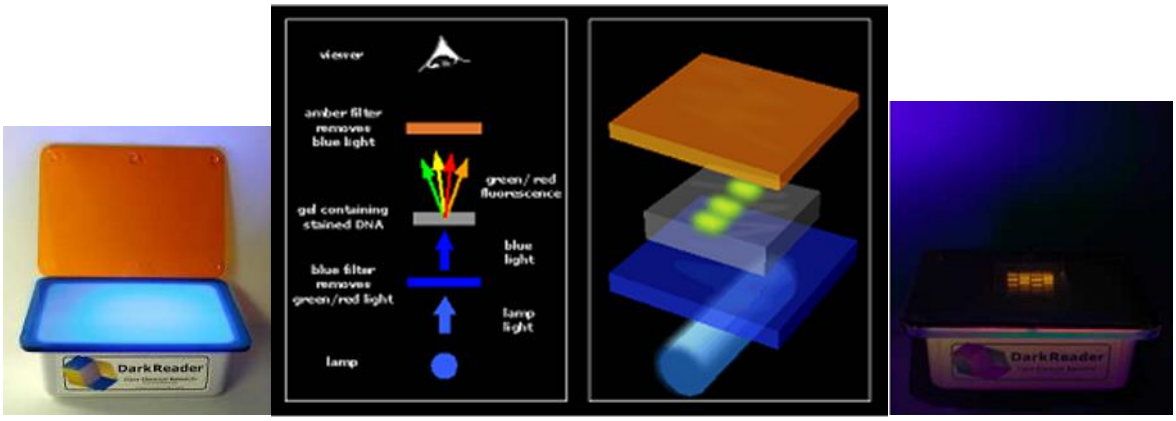
Role: determination of the size (length) of the DNA molecules

- The exact size can be only determined in case of linear or nicked circular DNA molecules
- The plasmids sometimes do not run at their exact size. Reason: coiling of circular DNA.
- Several DNA markers exist.
- The DNA marker should be appropriate to the size of the examined DNA molecule.
- The ladders already contain the loading dye; only the dye for visualization is needed to be added.
- Small amount of them are required, approx. 1,5-3 µl is enough for running.



Detection of DNA

- UV transilluminator- Excitation with UV light, UV filter!
- Transilluminator- excitation with blue light, amber screen absorbs the blue excitation light (orange filter)
- Gel documentation system – excitation with different wavelengths, detection of emission, gel photo



Questions:

1. What can affect the DNA run?

2. What is the role of the DNA ladder?

3. How can DNA be detected?

4. What can restriction endonucleases be used for?

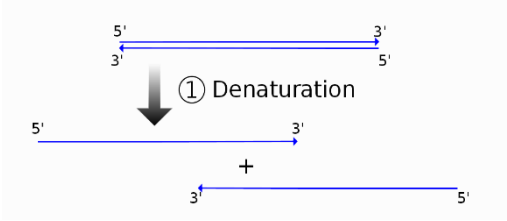
5. What is the principle of agarose gel electrophoresis?

5. PCR practice

Steps of polymerase chain reaction:

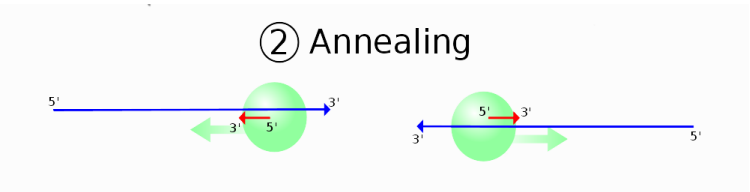
I. Denaturation

High temperature (92-98°C) denatures all the DNA.



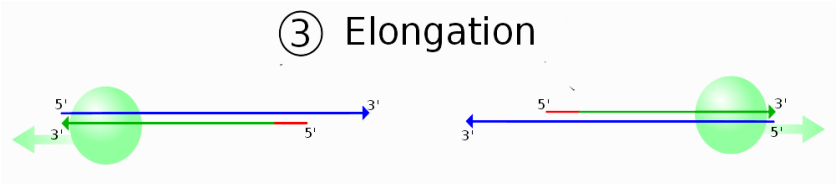
II. Annealing

Primer specific temperature (50-72°C) allows the oligonucleotides to anneal.



III. Elongation - Extension

The polymerase synthesizes the complement chain (60 or 72°C).

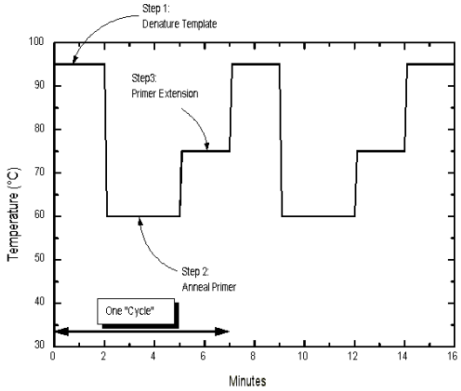


Components of the reaction:

- DNA template
- Primers
- Nucletotides (dATP, dGTP, dCTP, dTTP)
- DNA polymerase
- Buffer (including Mg^{2+})

Reaction heat profile:

92 °C 2’
92 °C 20”
56 °C 30” } 35x
72 °C 30”
72 °C 5’



Primers:

forward primer – human ß actin (Akt 11) 5’ AGA AAA TCT GGC ACC ACA CC 3’

reverse primer – human ß actin (Akt 21) 5’ GGG GTG TTG AAG GTC TCA AA 3’

Samples: negative control, positive control, sample 1 (HeLa cDNS), sample 2 (WRL68 cDNS)

Setting up the reaction:

1 reaction	**4 reactions (master mix)**
6 ul distilled water	… ul
10 ul 2x PCR buffer mix	… ul
2 ul forward + reverse primers	… ul
2 ul template	- ul
Σ 20 ul	Σ …. ul

Questions:

1. What can be the cause if we got products in the no template control reaction?

2. What can be the cause if we haven’t got product in the positive control reaction?

3. Why is it advised to use master mix?

4. Why do you need multiple repetitions?

5. What are the applications of PCR?

6. Cell culture practice

Cell culture is the process by which cells are grown under controlled conditions, generally outside their natural environment.

Extracorporeal growth of cells in vitro.

Based on Growth Mode

- Monolayer: sticky
- Suspension

Types of cell cultures

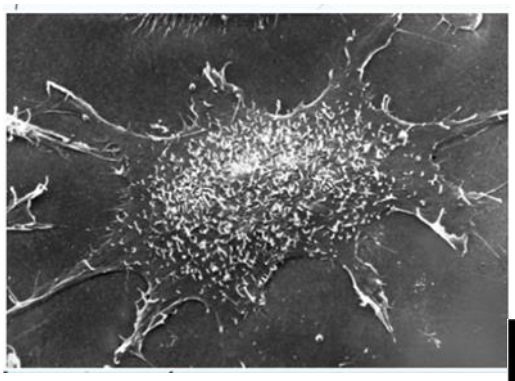
I. ***Primary cell culture****:* prepared from the original organ or tissue
   - only one type of cell presents in the culture
   - benefit: it shows the same fisiologycal conditions that exists in the living organism

II. ***Secunder cell culture****:* prepared after one passage of the primary culture

III. ***Cell strain****:* derived from primary culture after a few passage, but it has a finite division potential (40-50 passage).

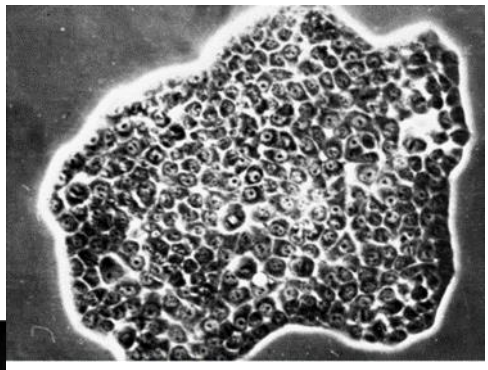
IV. ***Cell line****:* these cells have the ability to proliferate indefinitely, theoretically for unlimited time and are genetically homogeneous.
   - immortalization: the ability to proliferate indefinitely
   - transformation (onkogen transfection with retrovirus)
   - tumour cells (mutation or transfection with virus)
   - spontaneous immortalization

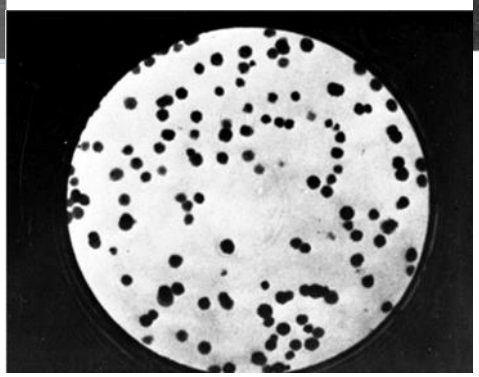
V. ***Cell banks****:* storage of cell strains and cell lines in a specific crioprotective media and liquid nitrogen.



One cell-SEM (0.01 mm)

Cell colony (1 mm)





Tissue culture plate with cell colonies

(100 mm)

Benefits of cell cultures:

- specific cells, homogenous cell population
- experiments are reproducible and planned correctly
- controlled conditions
- monitoring of cell functions (BUT! these results cannot be refered totally to the *in vivo* conditions)
- substitution some of the animal experiments
- replacing some of the animal experiments
- investigators of human cells

Limitations of Cell Cultures

- It does not model the complexity of the organization
- Spontaneous in vitro evolution
  - Spontaneous mutations arise from a variety of sources, including errors in DNA replication, spontaneous lesions, and other more complex mechanisms.
  - Spontaneous mutations are very rare, making it difficult to determine the underlying mechanisms.
  - Even though they are rare, some selective systems allow numerous spontaneous mutations to be obtained and then characterized at the molecular level for example, their DNA sequences can be determined.
  - From the nature of the sequence changes, inferences can be made about the processes that have led to the spontaneous mutations.

Maintaining of cell cultures

- STERILE work!
- **passaging***:* transferring a small and defined number of cells into a new vessel with fresh media.
- *Suspension cells*: easily passaged with a small amount of culture containing a few cells diluted in a larger volume of fresh media
- *Adherent cells*: cells first need to be detached, commonly with enzymatic digestion, then a small number of detached cells can be used to seed a new culture with fresh media
- number of passage is very important to memorize!

Materials needed for maintain cell cultures

a) **growth medium**: insures the right nutrients and conditions for cell growth and proliferation.
   - ions: Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, PO_4^{3-}, HCO_3^-
   - glucose: energy source
   - essential amino acids, that the cells cannot sythesize
   - vitamins
   - buffer: maintain pH 7,2-7,4

- phenol red: pH indicator, indicates the changes in pH balance
- serum: 5-20%, fetal: FCS/FBS or adult

b) **sterile bench:** avoid contamination with the filtration of the air

c) **cell incubator**: optimal temperature (37°C), 5% CO_2, optimal humidity (90-100%)

d) **microscope:** usually inverz

- Bürker chamber: to determine the number of living cells with the help of a dye: tripan-blue (only the death cells will be blue)
- cell culture flasks, multiwell plates, Petri-dishes, sterile pipettes and cetrifuge

Storage of cells

- in icing ampule and in specific icing media (to avoid the formation of ice crystals)
- *short-term storage*: -80°C
- *long-term storage*: liquid nitrogen -196°C

Video – cell culturing

http://youtu.be/7d_kDu-P964

https://www.thermofisher.com/hu/en/home/global/forms/cell-culture-basics/cell-culture-basics-virtual-lab.html

Questions:

1. What are primary cell cultures? What is the advantage of a primary culture over a secondary culture?

2. What are the characteristics of cell lines?

3. What is typical for in vitro studies?

4. Why is the medium important for maintaining cell culture?

5. What does passaging mean?