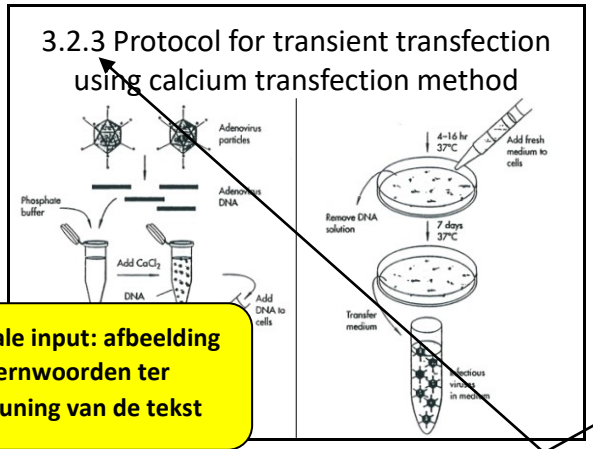
***Good practice*: syllabus cytologie Inge Maertens– opleiding biotechnologie – VIVES, campus Roeselare**

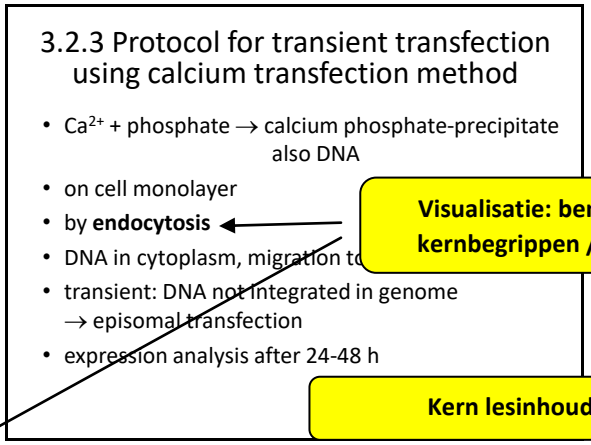
ILLUSTRATIE 1

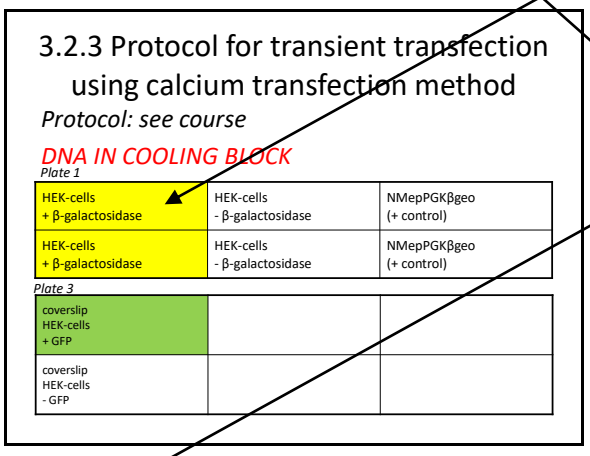
Inhoudsopgave

ILLUSTRATIE 2

Powerpoint-presentatie die hoort bij syllabus (onderdeel 3.2.3)







Visualisatie: benadrukken kernbegrippen / structuur

Multimodale input: afbeelding met kernwoorden ter ondersteuning van de tekst

Kern lesinhoud

Structuur bieden

3.2.3 Protocol for transient transfection using the calcium phosphate transfection method

Structuur bieden: titels en tussentitels, met nummering

3.2.3.1 Principle

When calcium and phosphate are mixed, in the presence of the DNA to be transfected, a calcium phosphate precipitate is formed under the right conditions, in which the DNA is coprecipitated. This precipitate is placed on the cell monolayer (60-70% confluence), which induces the contact of the precipitate with the cell membrane endocytosis. The DNA is released in the cytoplasm and migrates to the nucleus, where transcription starts.

Eenvoudige zinsbouw

In transient transfection, the absorbed DNA is not integrated into the genome of the cell, but is broken down after a few days or lost during cell division (episomal transfection). The expression analysis is therefore performed shortly after the transfection (24 to 48 hours after the transfection).

Ca-phosphate method is used for both stable and transient transfection.

3.2.3.2 Material

Gebruik van opsommingen om doorlopende tekst te doorbreken

- Growing medium
- Tips 10 – 200 – 1000 µl
- Micropipette
- 10 ml disposable pipette
- Microcentrifuge tubes 1,5 ml
- $CaCl_2$/hepes
- 0,1x TE
- 2x BS / 1x Hepes

The following solutions may be created at one time for the entire group.

$CaCl_2$/hepes (10 x concentrated) – 50 ml

				weight
• $CaCl_2$	1,25	mol/L	M 111	… g
• Hepes	125	mmol/L	M 238,3	… g

Adjust to pH 7,05 with NaOH.

Filter sterilization through a 0,22 µm filter.

TE – 100 ml	**mmol/l**	**RMM**	**weight g**
• Tris-HCl (pH 8,0)	1,0	121,14	…
• EDTA	0,1	292,25	…

Filter sterilization through a 0,22 µm filter.

BS / Hepes – 50 ml	**g/l**	**weight g**
• Hepes (25 mmol/L)	5,96	…
• NaCl	16,0	…
• KCl	0,74	…
• $Na_2HPO_4.12H_2O$	0,50	…
• Dextrose (D-glucose)	2,0	…

Adjust to pH 7,05 with NaOH.

Filter sterilization through a 0,22 µm filter.

3.2.3.3 Method

Note: In order to work scientifically correctly, wells with negative control should also be refreshed.

Cell line: ……..……

Plasmid: ……

1-5 µg DNA = ... µl DNA

Belangrijke gegevens vet gedrukt

Stapsgewijze opbouw

- Observe the cells under the microscope and estimate the % confluent cells (.... %).
- Change the medium of the wells being transfected: remove all medium and place 1,8 ml of medium per well in the 6 well plates.
- Incubate for minimum 1 hour at 37 °C.
- Create a mixture of **1-5 µg of plasmid DNA** = ... µl and dilute with ..... µl TE to 100 µl in a microcentrifuge tube of 1,5 ml.
- Add **25 µl $CaCl_2$/hepes**.
- Add this mixture under a sterile airflow to 125 µl of BS/hepes in a 1,5 ml microcentrifuge tube (see demonstration lecturer).
- Shake this assembly by hand for **1 min** and then centrifuge briefly afterwards.
- Add the **250 µl** to one 6-well (spread over the entire well).
- Cells are incubated until testing at 37 °C in the CO_2 incubator.

Note

For the transfection that needs to be carried out twice: double the quantity and add 240 µl per well to transform.

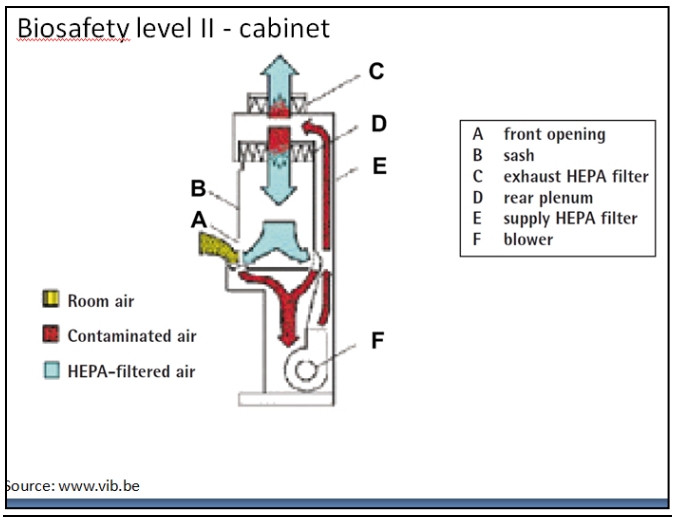
English words

Focus op taal: omschrijving begrip in het Engels + voorbeeldzin

to precipitate	Cause (a substance) to be deposited in solid form from a solution. *'cell proteins were then precipitated and washed in 10% trichloroacetic acid'*
to estimate	Roughly calculate or judge the value, number, quantity, or extent of. *'Experts estimate that between 70 % and 80 % of wireless networks are insecure.'*

ILLUSTRATIE 3

Powerpoint-presentatie die hoort bij syllabus (onderdeel 3)



Begrippen duiden door te visualiseren

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3 Stable and transient transfection of plasmid DNA in eukaryotic cells

For the work with NMf dells (Normal Murine mammary gland – fibroblastic cells) and NMe cells (Normal Murine mammary gland – epithelial cells) an L1 zone is sufficient. An L2 zone is required for working with HEK cells (Human Embryonic Kidney 293-cells). Hence, the entire lab will work as a (temporary) L2 zone. The 'Safety information guide in the lab' is brought along and the safety measures are respected.

Biosafety Level 1 (BSL-1)

BSL-1 is the basic level of protection common to most research and clinical laboratories, and is appropriate for agents that are not known to cause disease in normal, healthy humans.

Biosafety Level 2 (BSL-2)

BSL-2 is appropriate for moderate-risk agents known to cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure. Most cell culture labs should be at least BSL-2, but the exact requirements depend upon the cell line used and the type of work conducted.
(Gibco, Cell culture basics handbood, 2016).

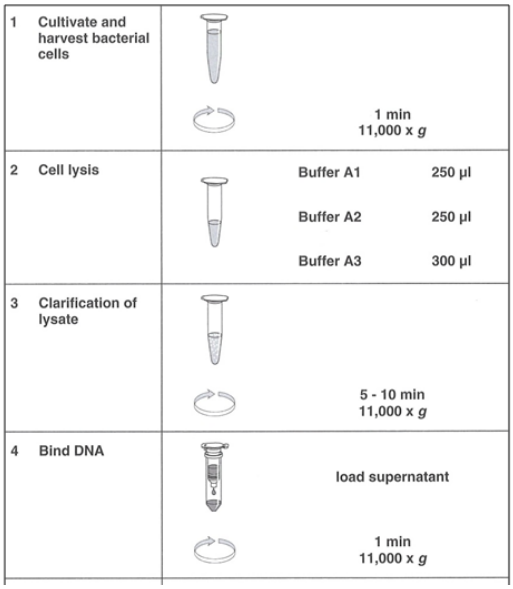
Focus op taal: omschrijving begrip in het Engels

English words

murine	Relating to or affecting mice or related rodents
mammary gland	The milk-producing gland of women or other female mammals

ILLUSTRATIE 4

Powerpoint-presentatie die hoort bij syllabus



Schematische voorstelling van de syllabus

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1. **Cultivate and harvest bacterial cells**
   Use **1-5 ml** of a saturated ***E. coli* LB culture**, pellet cells in a standard benchtop microcentrifuge for **30 sec** at **11 000 x g**. Discard the supernatant and remove as much of the liquid possible.

   Note
   In order to obtain more yield, the following procedure is used.
   Mix *E. coli* well with the LB medium. Place the culture in a 2 ml microcentrifuge tube. Centrifuge for 1 min at full force, discard the supernatant.
   Repeat this again 2 times.
   Use the pellet further in step 2.

2. **Cell lysis**
   Add **250 µl Buffer A1**. Resuspend the cell pellet completely by pipetting up and down. Make sure no cell clumps remain before addition of Buffer A2!

   Attention: Check Buffer A2 for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30-40 °C until precipitate is dissolved completely. Cool buffer down to room temperature (20-25 °C).

   Add **250 µl Buffer A2**. Mix gently by inverting the tube **6-8 times**. Do not vortex to avoid shearing of genomic DNA. Incubate at **room temperature** for up to **5 min** or until lysate appears clear.

ILLUSTRATIE 5