Bogomoletz Institute of Physiology

Manual for HEK-293 cell culture management

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December 2019

Content

1. Introduction

Read this document from the start to the end before starting doing something. If you lack understanding – ask your supervisor. Don't do premature optimizations – first 1 or even 2 times follow manual and the next time decide where you can cut costs.

Check and monitor local availability of consumables, solutions, etc. Delivery of new resources may take up to few months – make requests beforehand.

2. Solutions

There are some solutions required for HEK-293 cells, which are described below.

2.1. FBS

Fetal Bovine Serum – is an addition to the cell medium that provides proteins required for cell survival and growth. FBS is retrieved from animals and thus its content may vary between batches. Use another supplement if possible.

2.1.1. Storage

FBS needs to be stored in a freezer ($t \leqslant -5\,^{\circ}\mathrm{C}$). Storage in a fridge is possible for a short time (2 weeks) until sediment appears (degraded proteins, etc) which is a sure indicator of expiration.

Warning

Sediment in FBS is a sure indicator of expiration

Freezing/unfreezing cycles negatively affect FBS.

Warning

Do not repeat freezing/unfreezing of FBS more than 4 times

2.1.2. Aliquotes

Due to a low tolerance of FBS to freezing it's recommended to optimize managing of aliquotes, for example see fig. 2.1. When putting aliquotes into a freezer ensure they are placed vertically, so that the forming ice will not tear up the tube cap. This can be achieved with the tube rack. Choose faster freezing over slow to prevent possible stratification (for example, use the $t \leqslant -40\,^{\circ}\mathrm{C}$ freezer when freezing, then put for storage into any other freezer).

2.1.3. Before use

If FBS is frozen – unfreeze it in the fridge ($t \approx 5\,^{\circ}\mathrm{C}$) slowly ($\approx 2\,\mathrm{hrs}$ for $10\,\mathrm{mL}$ aliquot). Check for a uniform color and use the vortex mixer to ensure homogeneous content of the solution.

2.2. Trypsin

Trypsin-EDTA solution – is a specialized solution used to detach cells from a cover glass or other surface and destroy the connections between cells. It may have an indicator added that normally has pink color.

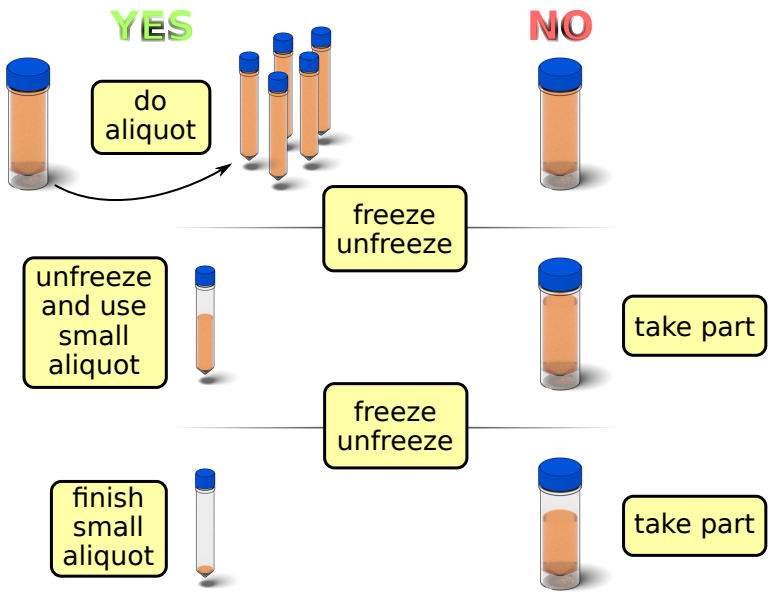

Fig. 2.1. Example of aliquot optimization for reducing the number of freezing cycles

2.2.1. Storage

Trypsin-EDTA needs to be stored in the freezer ($t \leqslant -5\,^{\circ}\mathrm{C}$). Storage in a fridge is not recommended. Yet it actually can work well after a month of being stored in a fridge ($t \approx 5\,^{\circ}\mathrm{C}$).

Freezing/unfreezing cycles negatively affect Trypsin-EDTA.

Warning

Do not repeat freezing/unfreezing of Trypsin-EDTA more than 4 times

2.2.2. Aliquotes

Due to a low tolerance of Trypsin-EDTA to freezing it's recommended to optimize managing of aliquotes, for example see fig. 2.1. Another important thing about the use of aliquotes is to keep the last one or a few aliquotes from the old batch, and instead use one or a few from the new batch fig. 2.2. If the new batch is faulty – you'll still have one or a few aliquotes of an old one to continue working while investigating the reason and looking for a solution.

2.2.3. Before use

Handling of trypsin-EDTA aliquotes is quite similar to FBS (see 2.1.3). But it must be warm before being applied to cells for the best result (see 4).

Note

Trypsin is like a human – it's not working when it has a room temperature

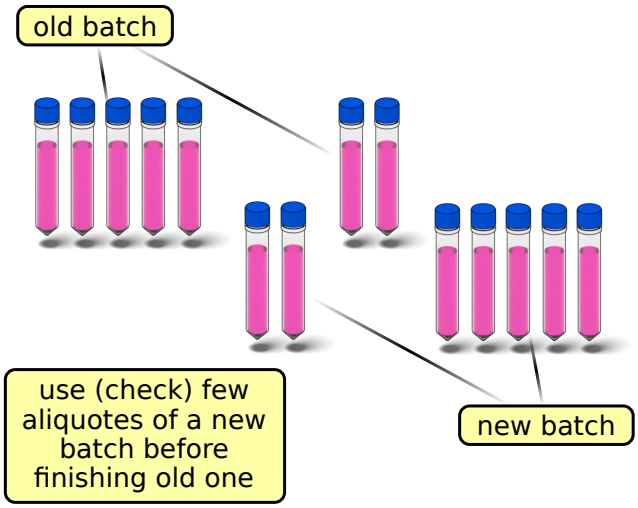

Fig. 2.2. Keep a few old aliquotes for the backup before you're sure that the new one is ok

2.3. DMEM

Dulbecco's Modified Eagle Medium is a base ingredient for cell growth environment. Do not mistaken it with DMEM+, a regular DMEM with additions (see 2.4).

2.3.1. Storage

DMEM must be stored in a fridge as aliquotes (see 2.3.2). DMEM degrades when exposed to the air by becoming alkaline which can cause damage to cells. Degrading can be seen as a change of DMEM color, which is due to a chemical indicator that is added. Crimson liquid is good, yellow – acidic, purple – alkaline (see fig. 2.3). Alkaline degrading can be partially undone by placing alkaline DMEM in atmosphere with $5\,\%$ of CO_2.

Warning

If DMEM without cells was purple (alkaline) and suddenly becomes yellow (acidic) – check for murkiness, most probably DMEM was contaminated with bacteria or fungi (see fig. 2.3). Throw it into a specialized trashcan and take actions to eliminate possible contamination at a workplace

Note

If DMEM was contaminated with fungi and you see single "cloud"in the tube and no black dots (spores), you "may"(better just throw out this aliquot as fast as possible) try to remove this fungi body with forceps in a special place away from the clean stuff, then check if DMEM is clear by placing a tube in $+37^{\circ}\mathrm{C}$ for a few days. Still, estimate the risk of losing a single aliquot of DMEM versus getting fungi contamination at a workplace.

Fig. 2.3. Example of DMEM colors with corresponding conditions that cause color change

2.3.2. Aliquotes

DMEM is often sold as $500\,\mathrm{mL}$ liquid in a bottle. As DMEM will degrade when exposed to air it's recommended to make aliquotes of the required amount (see fig. 2.4. It's a good idea to choose the right capacity of the container for the aliquot, for example, store $45\,\mathrm{mL}$ in the $50\,\mathrm{mL}$ tube, so you'll be able to add the other ingredients to this tube later and get DMEM+ (see 2.4) without a need to use the new tube. A big bottle of $500\,\mathrm{mL}$ (bottle may contain slightly more liquid) contains $11\times45\,\mathrm{mL}$ aliquotes, leftovers can be put in $5\,\mathrm{mL}$ tubes (a small amount of DMEM is needed for a transfection, see **??**). Aliquotes also reduce damage done by negligence. It's better to throw away one spoiled aliquote than the whole bottle. When preparing aliquotes never fill it from the bottleneck, always use big pipettes and the appropriate sampler. Don't forget to put notes with permanent marker on aliquotes with: a catalog number of DMEM; a date received; a date aliquoted; an amount of the liquid; the name of the person who made aliquotes; etc.

Warning

A neck of the big bottle has a huge chance to become contaminated, avoid spilling liquids from the bottleneck, always use appropriate pipettes and samplers

2.4. DMEM+

Dulbecco's Modified Eagle Medium with additions is a medium used for cell growth.

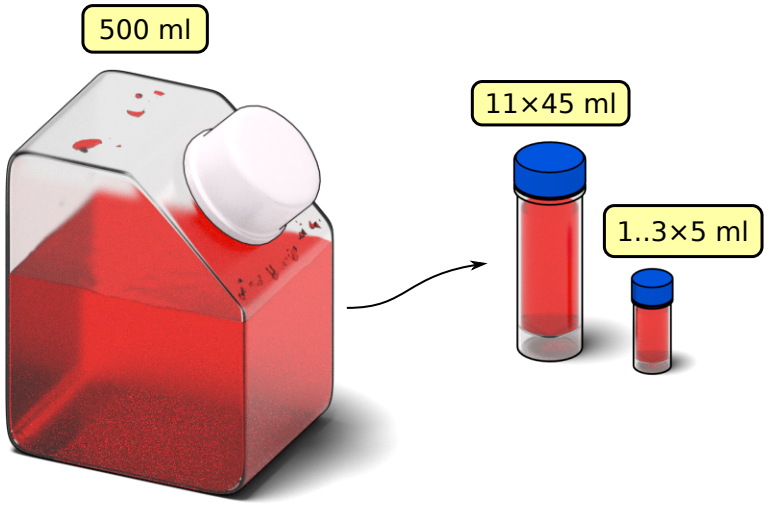

Fig. 2.4. Aliquotes from $500\,\mathrm{mL}$ of DMEM

2.4.1. Preparation

A portion of $50\,\mathrm{mL}$ of DMEM+ is prepared using the following components, see table 2.1 and fig. 2.5, notice that you can take a $10\,\mathrm{mL}$ aliquot of FBS and remove $5\,\mathrm{mL}$ then put it back into freezer and use the next time.

Protocol:

1. Preparation
   1.1. Ensure the workplace is clean, there is a distilled water for washing tools and glass, and there is a $70\,\%$ ethanol in the water solution in a sprayer
   1.2. Check if all aliquotes are available (see. table 2.1 and fig. 2.5). You need to pick one antibiotic, don't use both

Components for DMEM+ preparation Table 2.1

Ingredient	Amount	Description
DMEM	$45\,\mathrm{mL}$	Base ingredient
FBS	$5\,\mathrm{mL}$	see 2.1
Gentamicin	$1{,}25\,\mathrm{mg}$	Antibiotic, dissolved in the liquid with known concentration $10..50\,\mathrm{g/L}$
(optional) Pen&Strep	$300\,\mu\mathrm{L}$	Antibiotic

   1.3. Check for required equipment, tools, etc (see table 2.2 and fig. 2.6)
   1.4. Unfreeze FBS (refer to 2.1.3), this can take few hours
   1.5. Turn on the Biological Safety Cabinet (BSC) and put there tools and equipment (see table 2.2 and fig. 2.6)
2. Making

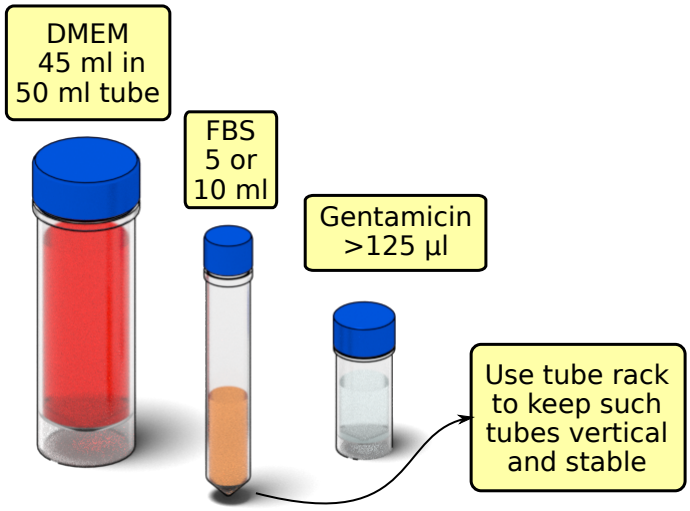

Fig. 2.5. Aliquotes required to prepare DMEM+

Tools and equipment for DMEM+ preparation Table 2.2

Tool	Description	Notes
Pipettes	$200\,\mu\mathrm{L}$; $1\,\mathrm{mL}$; $5\,\mathrm{mL}$	
Tips for pipettes	$200\,\mu\mathrm{L}$; $1\,\mathrm{mL}$; $5\,\mathrm{mL}$	
Glass for trash	For used tips, etc	must be empty and clean
Tube rack	Holds aliquotes vertically	
Laboratory film	Parafilm, used to seal tubes	
Permanent marker	For notes on tubes, etc	
Sterile gloves		Put them the palm side up when doing UV sterilization

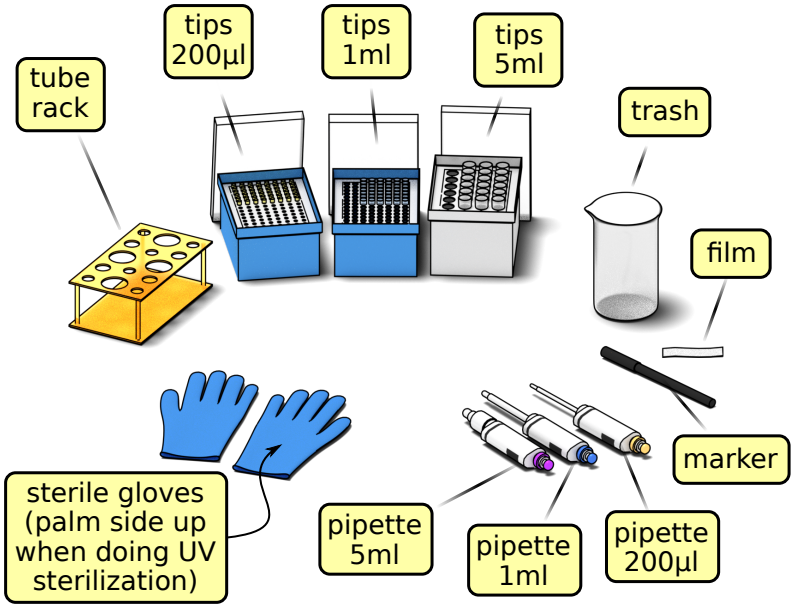

Fig. 2.6. Tools and equipment required for DMEM+ preparation

2.1. Start the BSC sterilization ($15..20\,\mathrm{min}$ UV exposure, **DO NOT** stare at the UV emission light even trough the glass) and wait till it's done

2.2. Get required solutions out of the fridge (a vortex may be needed, refer to the section for corresponding ingredient)

2.3. Tubes with solutions can't be sterilized with UV as it will damage solutions, so put them on a piece of a clean paper towel, spray with a $70\,\%$ ethanol in the water solution then wipe them with this paper towel and put into the BSC

2.4. Use a $5\,\mathrm{mL}$ pipette and the appropriate sampler to add $5\,\mathrm{mL}$ of FBS to $45\,\mathrm{mL}$ of DMEM (a tube should be of $50\,\mathrm{mL}$ capacity, see 2.3.2)

2.5. Then use a $200\,\mu\mathrm{L}$ pipette and the appropriate sampler to add $125\,\mu\mathrm{L}$ of Gentamicin

2.6. Mix the contents using a $5\,\mathrm{mL}$ pipette and the sampler with $15..20$ intakes/outtakes.

2.7. Close the tube and mix by swirling the contents of the tube. Do not shake it hard, as it may make a lot of bubbles and some of the liquid will be stuck at the cap which can later cause easy contamination

2.8. If you don't need it now then seal the edge between a cap and a tube with a piece of the laboratory film (to prevent the leak of the $\mathrm{CO_2}$ from internal atmosphere)

2.9. Put markings on this aliquot: the name of a solution (DMEM+), your name, a date, notes (for example, DMEM was expired $25\,\mathrm{years}$ ago)

2.10. Use this DMEM+ aliquot now or put it in the fridge for storage

2.11. Clean up and leave

3. Cultivating cells

3.1. Store

HEK-293 cells are normally stored inside the incubators with $37\,^{\circ}\mathrm{C}$ and $5\,\%$ of CO_2. Thus containers for this cells must have ventilation capabilities to ensure the gas exchange. There are many types of containers for a cell storage and growth that vary by form, area, venting type, the possibility to place a cover glass (which later could be used in the experiment, see 3.3) etc. Some of them are listed in the table 3.1 and fig. 3.1.

Containers for HEK-293 storage and growth Table 3.1

Name	Preferred area	Venting	Optimal liquid volume	Cover glass	Notes
Plastic petri dish	$\varnothing 35\,\mathrm{mm}$	Cap is not tight	$2\,\mathrm{mL}$	Yes, No	Low height is bad as medium can spill and cause contamination. There are also glass types of petri dishes
Plastic flask	$25\,\mathrm{cm}^2$	Cap either has semi-open state or has holes with a filter	$5\,\mathrm{mL}$	No	Preferred for long term storing ($> 5\,\mathrm{days}$). Cap without holes is better as it allows to make the flask hermetic when taking it out of the incubator
Plastic Multidish	12 wells $3{,}5\,\mathrm{cm}^2$ each	Lid is not tight	$1\,\mathrm{mL}$ per well	Yes, $\varnothing 18\,\mathrm{mm}$	Preferred for short time growth of cells on a cover glass for the experiment. Use one well only once. Remove liquid when taking the glass out of a well to avoid contamination

3.2. Feeding

HEK-293 cells require a change of the medium (DMEM+) once in a while (full replace). It's optimal to keep the DMEM+ level at a height of $2..3\,\mathrm{mm}$. Normally medium change should happen each $2..4\,\mathrm{days}$ depending on cell density as shown on fig. 3.2. Ensure that cells are not contaminated fig. 3.3.

Warning

If cells are contaminated immediately take them together with a container and throw into a specialized trashcan. Then take actions to remove contamination in the incubator and workplace

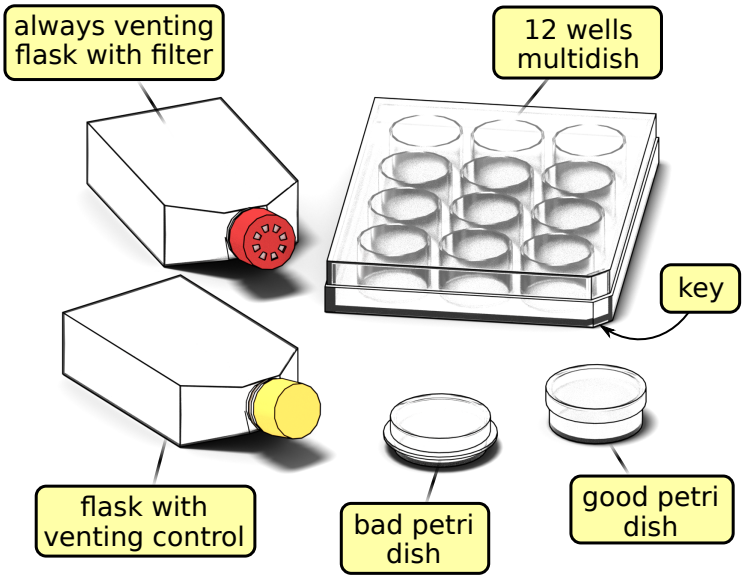

Fig. 3.1. Some types of containers for HEK-293 cells

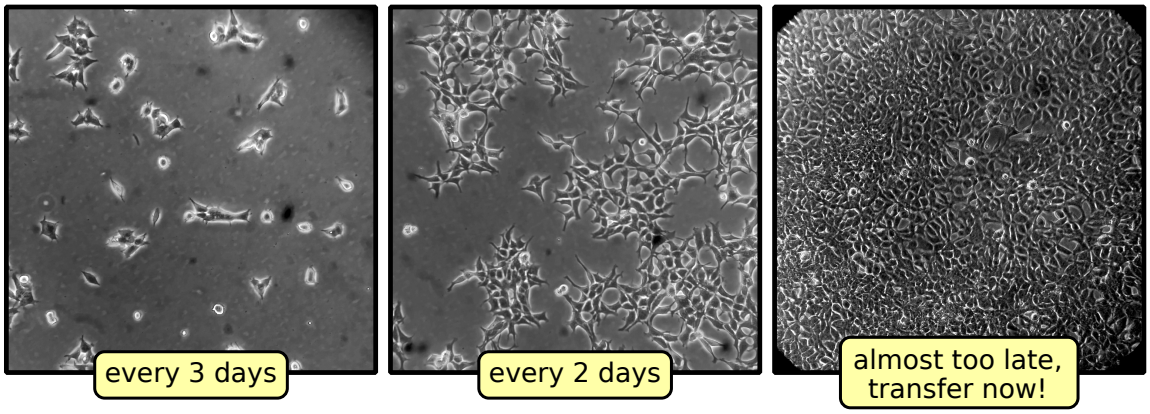

Fig. 3.2. Feeding rate depending on cell density

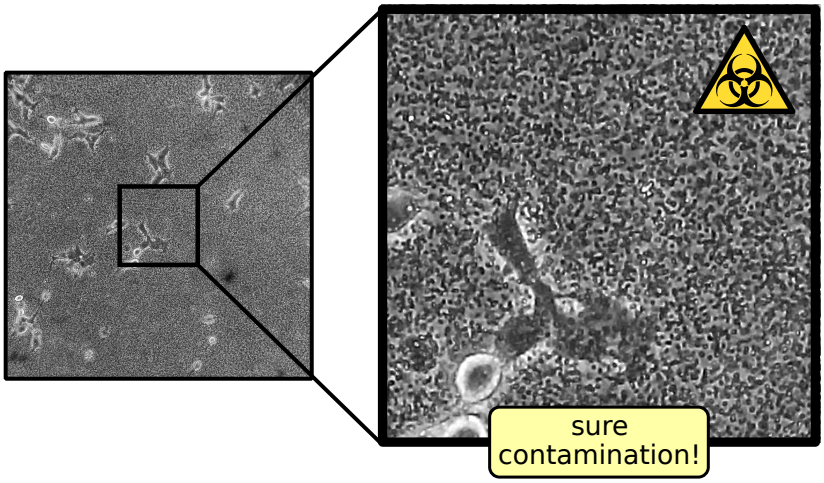

Fig. 3.3. Contaminated cells – take action immediately

3.3. Cover glass

Placing HEK-293 on the cover glass for the later experiments is done during the cell transfer (see 4). However, cover glasses must be cleaned and sterilized beforehand. For a multidish with $3{,}5\,\mathrm{cm}^2$ wells it's optimal to use round cover glasses with $\varnothing 18\,\mathrm{mm}$. Preparing a cover glass is done by washing them in distilled water (both sides) and then sterilizing in the thermostat for at least $1\,\mathrm{hour}$ at $180\,^{\circ}\mathrm{C}$ as shown on fig. 3.4. The cover glasses can be considered sterile for a few days when stored at a clean place inside a glass petri dish that is wrapped with foil.

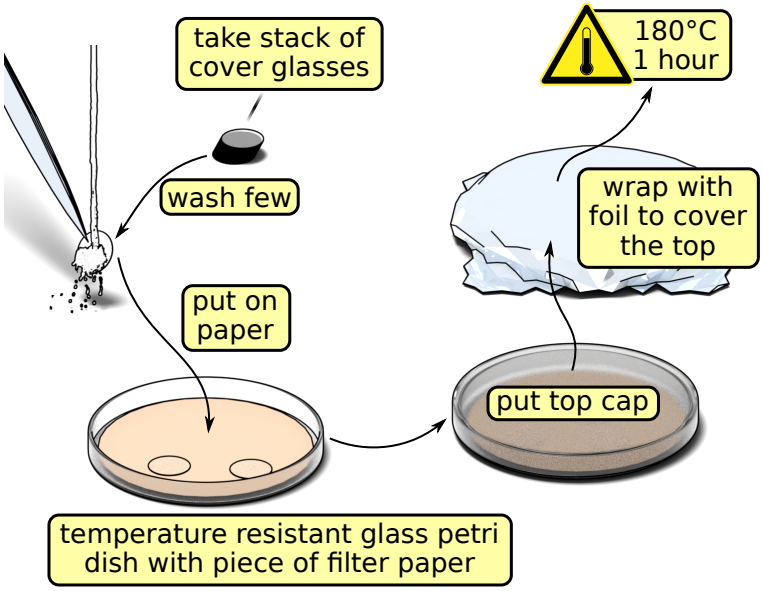

Fig. 3.4. Washing and sterilizing cover glasses before the use

4. Transfering cells

During the transfer cells detach from the current container and are placed into the new one. Transferring is important since cells become too numerous and start dying. They fill up the container and some of them stop receiving nutrients and die, releasing the substances that induce further cell death. It's recommended to avoid the situation on the rightmost panel of fig. 3.2: cells already start dying, they will be alive in a new container, but there will be a lot of the debris from dead cells which negatively affects the ones alive. For a long term growth cells can be placed into a new container in small amounts. But too small amount of cells is bad as cells will still overmultiply at some areas as shown on fig. 4.1. Also cells might die if they don't have any close neighbors. Recommendations regarding the amount of cells that should be added to the new container are discussed further.

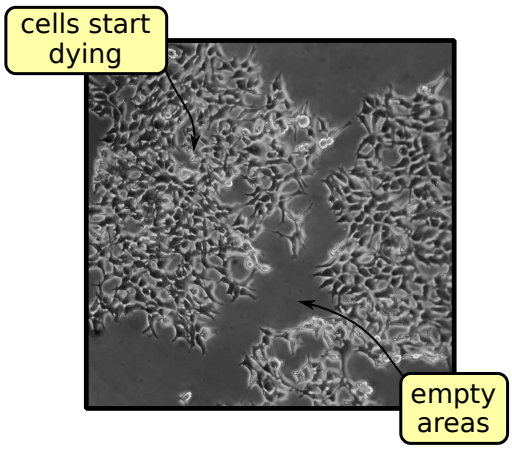

Fig. 4.1. Cells transfered in small amounts and still overmultiplied

A quick example of cell transfer from the old petri dish to the new one is given on fig. 4.2. The full protocol given below.

Protocol:

1. Preparation
   - 1.1. Ensure the workplace is clean, there is a distilled water for washing tools and glass, and there is a $70\,\%$ ethanol in the water solution in a sprayer
   - 1.2. Check if all aliquotes are available (see. table 4.1 and fig. 4.3). Preheat it in a thermostat for at least $20\,\mathrm{min}$.
   - 1.3. Check for the required equipment, tools, etc (see table 4.2 and fig. 4.4)
   - 1.4. Work in the gloves
   - 1.5. Turn on the Biological Safety Cabinet (BSC) and put there tools and equipment (see table 4.2). Don't forget to open containers as shown at fig. 4.4). Put the gloves into the BSC at last, obviously
2. Transfering

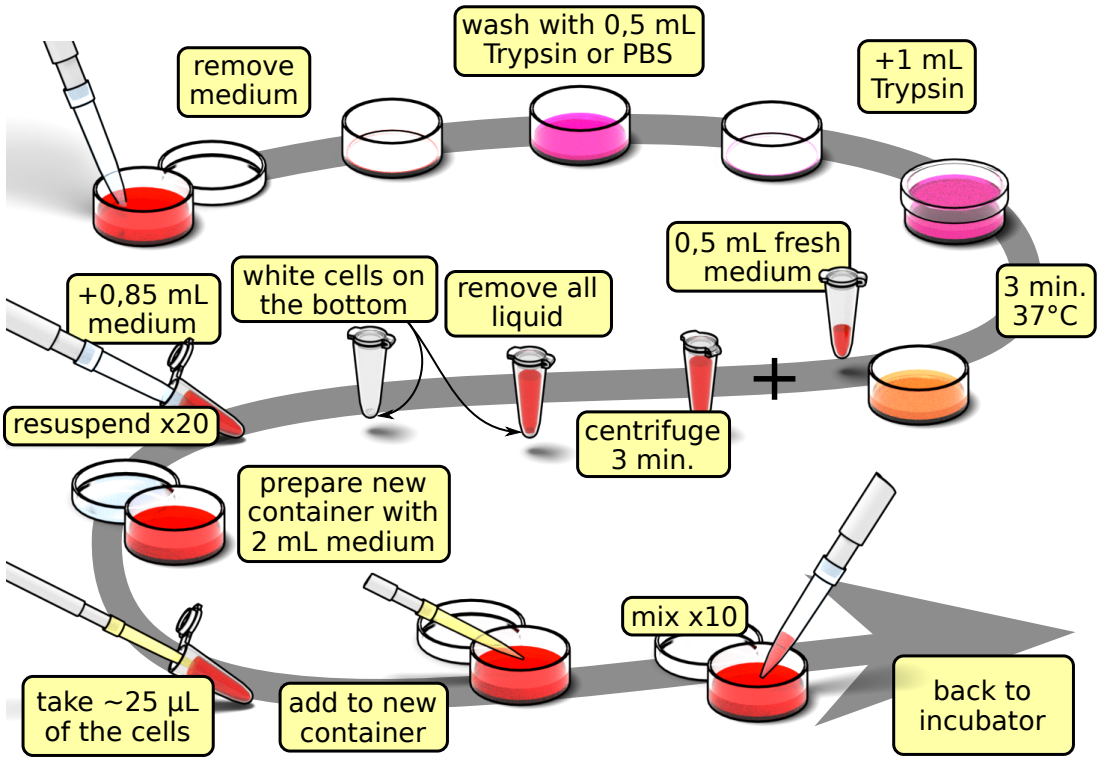

Fig. 4.2. A short protocol for cell transfer from petri dish

Solutions required to transfer cells Table 4.1

Solution name	Min. Amount	State	Description
DMEM+	$10\,\mathrm{mL}$	$+24..+37°\mathrm{C}$, crimson red liquid	
(optional) PBS	$1\,\mathrm{mL}$	$+24..+37°\mathrm{C}$, transparent liquid	**NOT** FBS, buffer solution used to wash cells of an old medium
Trypsin	$3\,\mathrm{mL}$	$+37°\mathrm{C}$, pink liquid	Used to detach cells from the bottom and destroy connections between cells, can be used instead of PBS. Sensitive to freezing/unfreezing cycles

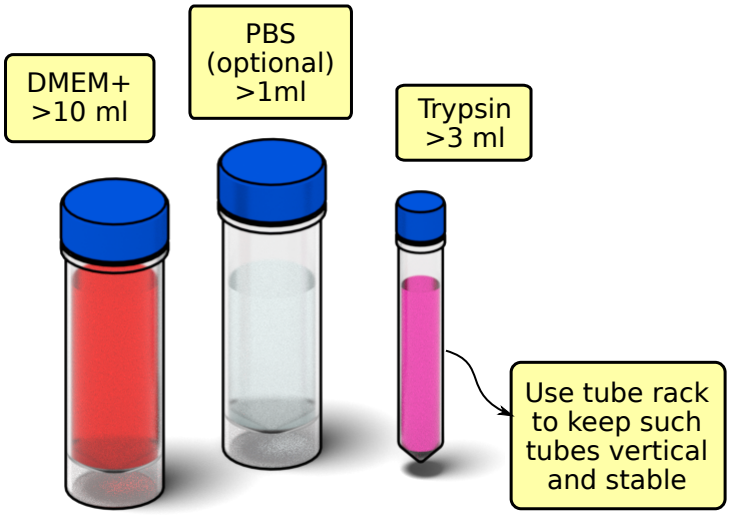

Fig. 4.3. Solutions required to transfer cells

Tools and equipment to transfer cells Table 4.2

Tool	Description	Notes
Pipettes	$200\,\mu\mathrm{L}$; $1\,\mathrm{mL}$; $5\,\mathrm{mL}$	
Tips for pipettes	$200\,\mu\mathrm{L}$; $1\,\mathrm{mL}$; $5\,\mathrm{mL}$	
1 or 2 tubes $1{,}5\,\mathrm{mL}$ with lock cap	Temporary storage for cells	
New sterile container	Flask or petri dish or multidish, etc	See 3.1
Glass for trash	For used tips, etc	must be empty and clean
Tube rack	Holds big tubes vertically	
Small tube rack	Holds small tubes vertically	
Box with cover glasses (optional)	If you plan experiment	See 3.3
Small forceps (optional)	To handle a cover glass	
Laboratory film (optional)	Parafilm, to refresh sealing of aliquotes	
Permanent marker	For notes on petri dishes, etc	
Sterile gloves		Put them the palm side up when doing UV sterilization

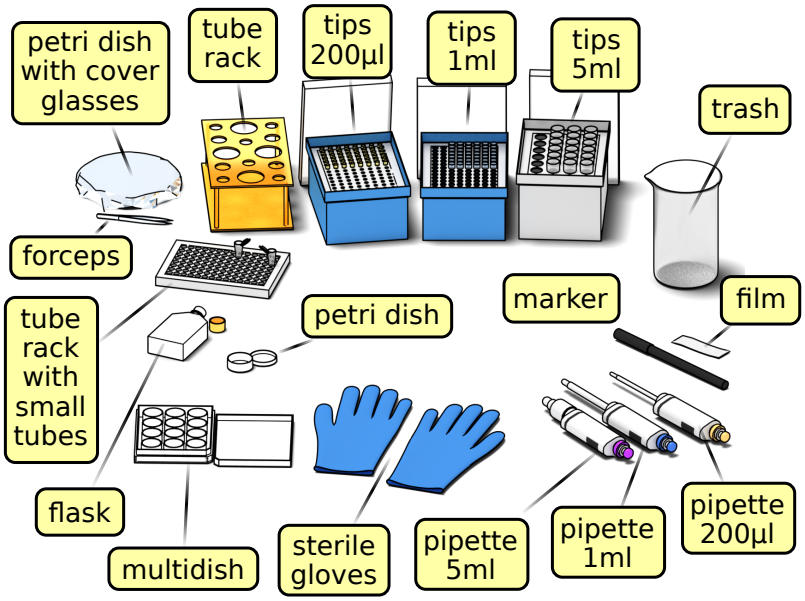

Fig. 4.4. The full set of tools required to transfer cells

2.1. Start the BSC sterilization ($15..20\,\mathrm{min}$ UV exposure, **DO NOT** stare at the UV emission light even trough the glass) and wait till it's done

2.2. Put the gloves on

2.3. Close all the containers

2.4. Get the required solutions (a vortex may be required, refer to a section for the corresponding solution)

2.5. Tubes with solutions can't be sterilized with UV as it will damage the solutions, so put them on a piece of a clean paper towel, spray with a $70\,\%$ ethanol in the water solution then wipe them with this paper towel and put into the BSC

2.6. Open the PBS (or Trypsin) aliquot

2.7. Open the old container and remove the old medium – use a $1\,\mathrm{mL}$ or $5\,\mathrm{mL}$ pipette and a sampler for petri dishes. For the flask just carefully pour the old medium out into a trashcan (don't scatter any drops elsewhere)

2.8. Add $1\,\mathrm{mL}$ of PBS (or Trypsin) to a petri dish or flask and wash the surface with cells for $5..15\,\mathrm{sec.}$ by gently swirling it

2.9. Remove this PBS (or Trypsin) from the petri dish with a $1\,\mathrm{mL}$ pipette and sampler, and just pour it out from a flask into a trashcan

2.10. Add $1\,\mathrm{mL}$ of Trypsin to the petri dish and $2\,\mathrm{mL}$ to the flask. Gently swirl the container (flask or petri dish) to ensure that trypsin covers the whole surface with cells. Further actions must be fast yet precise as Trypsin can destroy cells

2.11. Close the container. Put a cap on the petri dish, close the flask airtight (if possible) and put it into the incubator with $+37\,^{\circ}\mathrm{C}$ and $5\,\%$ of $\mathrm{CO_2}$ for $3\,\mathrm{min.}$

2.12. While cells are in the incubator add $0{,}5\,\mathrm{mL}$ DMEM+ into the two $1{,}5\,\mathrm{mL}$ tubes with locking caps and close them. It will deactivate the leftover Trypsin later

2.13. Take the container with cells and Trypsin out of the incubator and check if there is a visible sediment flowing on the bottom of the container (detached cells). Trypsin may become yellow. Optionally use the microscope to see if cells have actually detached. Swirl container gently to help cells to detach from the bottom. If cells are not detached – ask your supervisor, you may have a bad Trypsin or another problem

2.14. Use a $1\,\mathrm{mL}$ pipette and tip to put this suspension with cells from the container into that two small tubes ($2 \times 1\,\mathrm{mL}$ from the flask and $2 \times 0{,}5\,\mathrm{mL}$ from a petri dish)

2.15. Now you may move slower if needed. Close this tubes airtight (visually inspect a tubes cap, some tubes have bad locking) and put them in the centrifuge so that there is a mass balance (there should be some dull tubes with water to help achieving the mass balance) for $3\,\mathrm{min}$.

> **Warning**
>
> Unbalanced weight in the centrifuge can cause damage to the equipment and, for the bigger centrifuges, to people. Don't open lid or put your hands or anything into the centrifuge until it is fully stopped

2.16. Take that small tubes out of the centrifuge. Ensure they are not damaged (centrifuge can damage the weak tubes with liquid)

2.17. Check if there is a pressed white sediment in the bottom of tubes (cells) – normally about $5..20\,\mu\mathrm{L}$ by eye

2.18. Carefully (but quickly) remove the liquid above the pressed cells by using a $1\,\mathrm{mL}$ pipette and tip

2.19. Choose a tube with more cells, the second one is for reserve (later you can optimize your protocol to use only one tube)

2.20. Using a $1\,\mathrm{mL}$ pipette and tip add $850\,\mu\mathrm{L}$ of DMEM+ to the chosen tube

2.21. Re-suspend cells $20..25\times$ using the $1\,\mathrm{mL}$ pipette and tip carefully (rise and lower the tip to follow the liquid level to avoid producing bubbles and spilling liquid) yet fast enough (to ensure cells disperse well in liquid). Then close the tube

> **Warning**
>
> As a multidish may be used many times (until all wells are used) it may contain dirt in the old wells. So, if you need to use a multidish – deal with it **AFTER** you finished transfering cells to the new flask or petri dish to avoid contamination during the long term storage.

2.22. Take a new container. If needed put cover glasses using forceps there

2.23. Then add the required amount of DMEM+ (see table 3.1)

2.24. Use a $200\,\mu\mathrm{L}$ pipette and tip to add $20..200\,\mu\mathrm{L}$ of the suspension from that tube. This amount depends on how much cells were there at the beginning and what density you want to achieve. For the first time you can add $50..100\,\mu\mathrm{L}$ to the new petri dish or flask (or less to keep it for a longer time) and $150\,\mu\mathrm{L}$ to a well with a cover glass in the multidish (or more to be ready faster for the experiment)

2.25. For the petri dish or multidish mix by doing $10..20\times$ intakes/outtakes with a $1\,\mathrm{mL}$ pipette and tip (again, don't make bubbles)

2.26. Close the container

2.27. Mix some more by gently swirling the container

2.28. Put the markings on the top (some containers have place for markings): a date; a cell type (HEK-293); your name; notes.

2.29. Look at the container in the microscope to check cells. The example of different density is shown on fig. 4.5. Note that you need to count cells that that sit on the bottom and the still flowing ones. Alternatively, you may place the container to the incubator for $15\,\mathrm{min}.$ and then check it again, see fig. 4.6. HEK-293 cells with very low density don't feel well. In this case you may add more suspension with cells as described above.

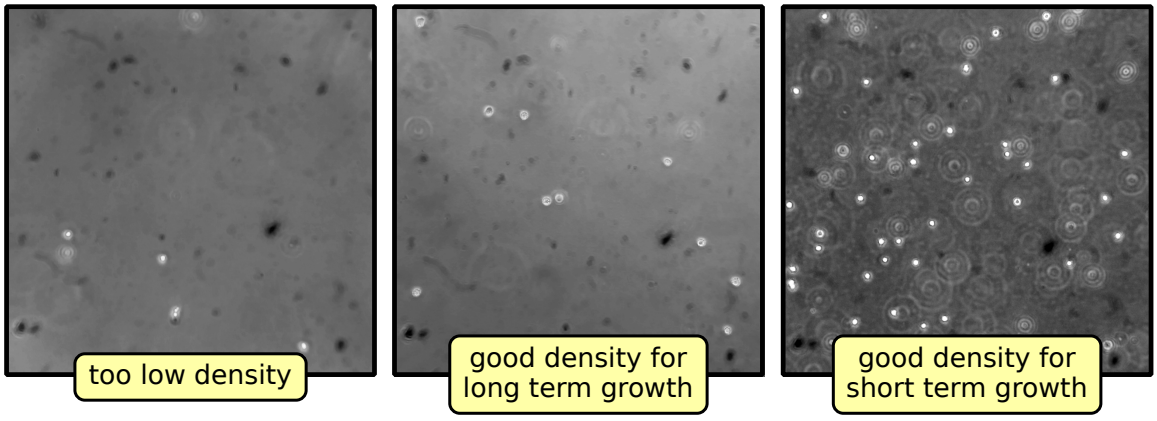

Fig. 4.5. HEK-293 cells recently placed into the new container

2.30. Place the new container in the incubator

2.31. If you still have to place cells in the multidish – repeat steps since 2.22

2.32. Ensure that all aliquotes are closed airtight and wrapped with the laboratory film. It's time to return them to the storage place

2.33. Clean up and leave

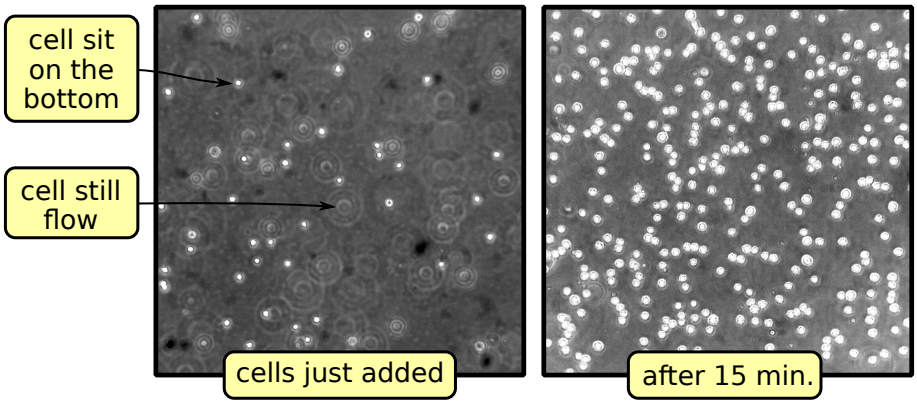

Fig. 4.6. Checking HEK-293 cells density right after adding cells and after 15 min.

5. Transfecting cells

HEK-293 cells are commonly used in biological sciences due to their high propensity to transfection – the procedure that makes cells produce the wanted protein by loading cells with DNA of that protein in a plasmid vector. Transfection procedure is described below.

For the experimental use it's best to grow cells on a cover glass (see 3.3) placed in a multidish (see 3.1). Cells divide in the main container, and then they fill it up and should be transfered to the new one (see 4). During this operation all cells are mostly taken from the old container and a few of them are placed in the new one. Large leftovers can be used to grow cells on a cover glass for experiments. This can be described with the following diagram fig. 5.1

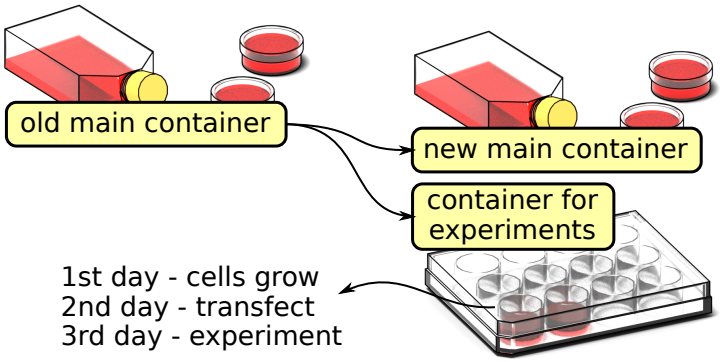

Fig. 5.1. Preparing separated cells for the transfection during transfering cells from the old container to the new one

Transfection is done by using plasmid DNA (construct) and special reagents. Good results for HEK-293 cell transfection gives Lipofectamine 3000 (refer to official manual). Plasmid DNA is normally diluted in the special medium. Concentration must be known and written on the plasmid container as $C = \frac{m[\mathrm{g}]}{V[\mathrm{L}]}$. Usually concentration is in the range of $C = 0{,}5..2\,\mathrm{g/L}$. The amount required for transfection is about $m = 0{,}25..2\,\mu\mathrm{g}$. The volume that should be added to achieve the required amount of DNA is calculated as $V = \frac{m}{C}$. Before preparing for the transfection ensure that required plasmids are available in necessary amount. If there is not much left ($< 5\,\mu\mathrm{g}$) – don't use it, ask supervisor about possible amplification.

Transfection can be used to infect cells with multiple different constructs. On practice the maximum is 2 constructs. In this case transfection can be done differently depending on the wanted result, either by separated transfection (some cells are infected with the first construct, others with the second, some with both, and some are not affected at all) or co-transfection (cells are transfected with both constructs or not affected). This is shown on fig. 5.2.

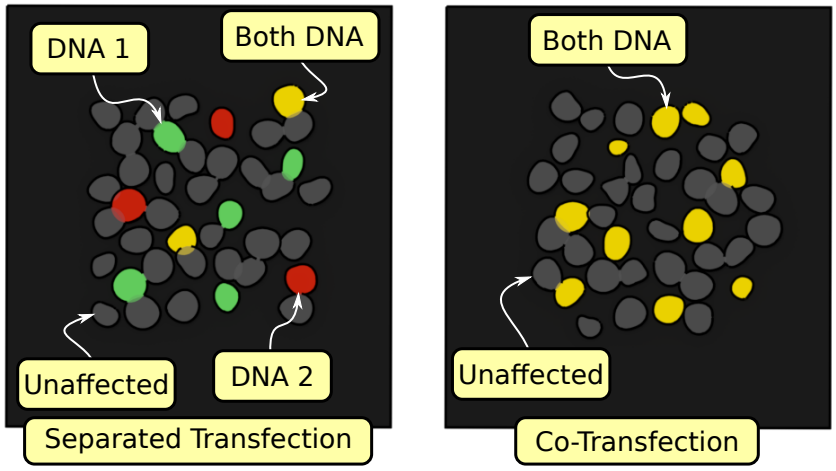

Fig. 5.2. Schematic example of cells transfected with separated transfection and co-transfection

1. Preparation

1.1. Ensure workplace is clean, there is a distillated water for washing tools and glass, and there is $70\,\%$ ethanol in water solution in sprayer

1.2. Check if all aliquotes available (see. table 5.1 and fig. **??**). Amount of required solution depend on how many cover glasses must be transfected and how many DNA plasmids should be used.

Solutions required for transfection per one cover glass $18\,\mathrm{mm}$ Table 5.1

Solution name	Min. Amount	State	Description
DMEM or OptiMEM	$1\,\mathrm{mL}$	$+24..+37^{\circ}\mathrm{C}$, crimson red liquid	
Lipofectamine 3000 «L» component	$1\,\mu\mathrm{L}$	$+24^{\circ}\mathrm{C}$	
Lipofectamine 3000 «P» component	$\frac{m_{\mathrm{DNA}}}{1\,\mu\mathrm{g}} \times 2\,\mu\mathrm{L}$	$+24^{\circ}\mathrm{C}$	$2\,\mu\mathrm{l}$ per $1\,\mu\mathrm{g}$ of plasmid
Plasmid DNA dispersed in special water	$> 5\mu\mathrm{g}$	$+24^{\circ}\mathrm{C}$	Don't use plasmid aliquote if nothing will left after, this may be the last plasmid sample you have

6. Catalog numbers

List of known tools, equipment, solutions, consumables, etc. Basic tools required to run biological laboratory is not listed here.

Table 6.1

Category	Name	Cat./Ref. №	Link
Consumables			
Petri dishes	CELL CULTURE DISH	627160	shop.gbo.com
Flask			
Multidish			
Solutions			
Medium	DMEM	41966029	thermofisher.com
Supplement	FBS	F9665	sigmaaldrich.com
Antibiotic	Gentamicin	15710064	thermofisher.com

7. Troubleshooting

This list contain some extra problems. For general problems refer to other sections of this manual. You can help by expanding this list

Troubleshooting Table 7.1

Problem	Possible reason	Possible solution
I've read one thing but nothing works	You didn't read whole manual before start doing something and everything goes wrong	Go read whole manual before you start
DMEM/DMEM+		
DMEM or DMEM+ is purple	DMEM has lost $\mathrm{CO_2}$ which cause change of pH and color of indicator that present in DMEM	Put open DMEM aliquot in atmosphere with $5\,\%$ of $\mathrm{CO_2}$
Un-caging		
NP-EGTA not working	NP-EGTA may react with Mg in inner solution and cause uncaging of $\mathrm{Mg^{2+}}$ instead of $\mathrm{Ca^{2+}}$	Prepare outer solution without Mg, that should decrease inner concentration of Mg

Nomenclature

BSC – Biological Safety Cabinet

DMEM – Dulbecco's Modified Eagle Medium

EDTA – Ethylenediaminetetraacetic Acid

FBS – Fetal Bovine Serum

UV – Ultra-Violet

References