КАЗАНСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ

2025г.

# Тема 12. Методы работы с клетками

лекция

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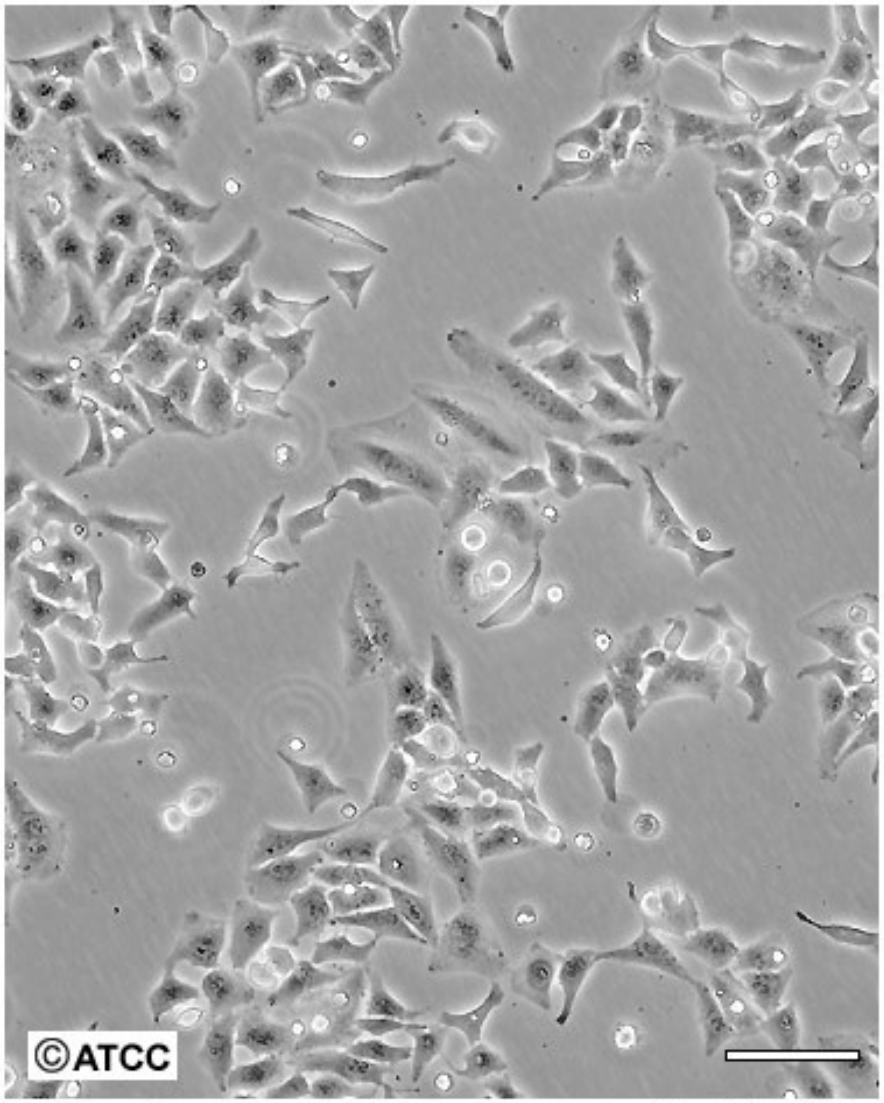
# Метод клеточных культур

# Основные типы клеточных культур

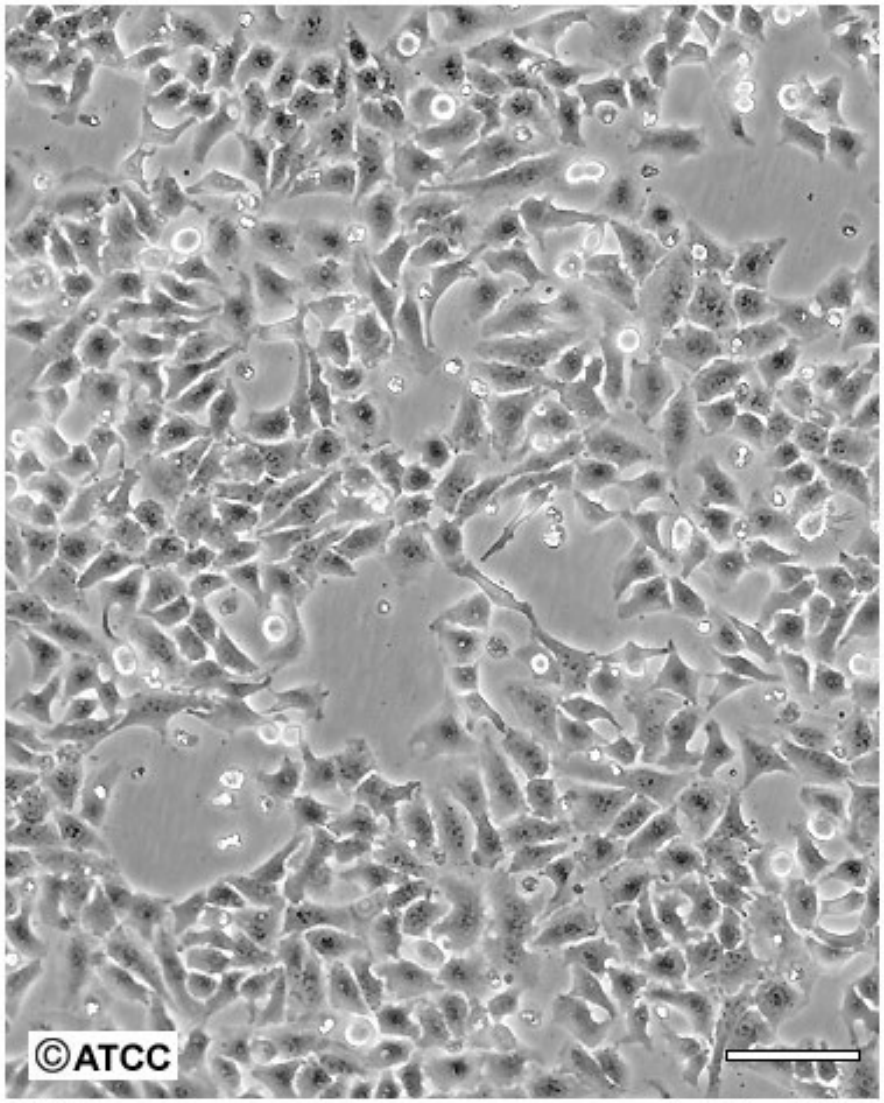
- Клеточные
- Тканевые

Designation: **HeLa**




Low Density

Scale Bar = 100µm

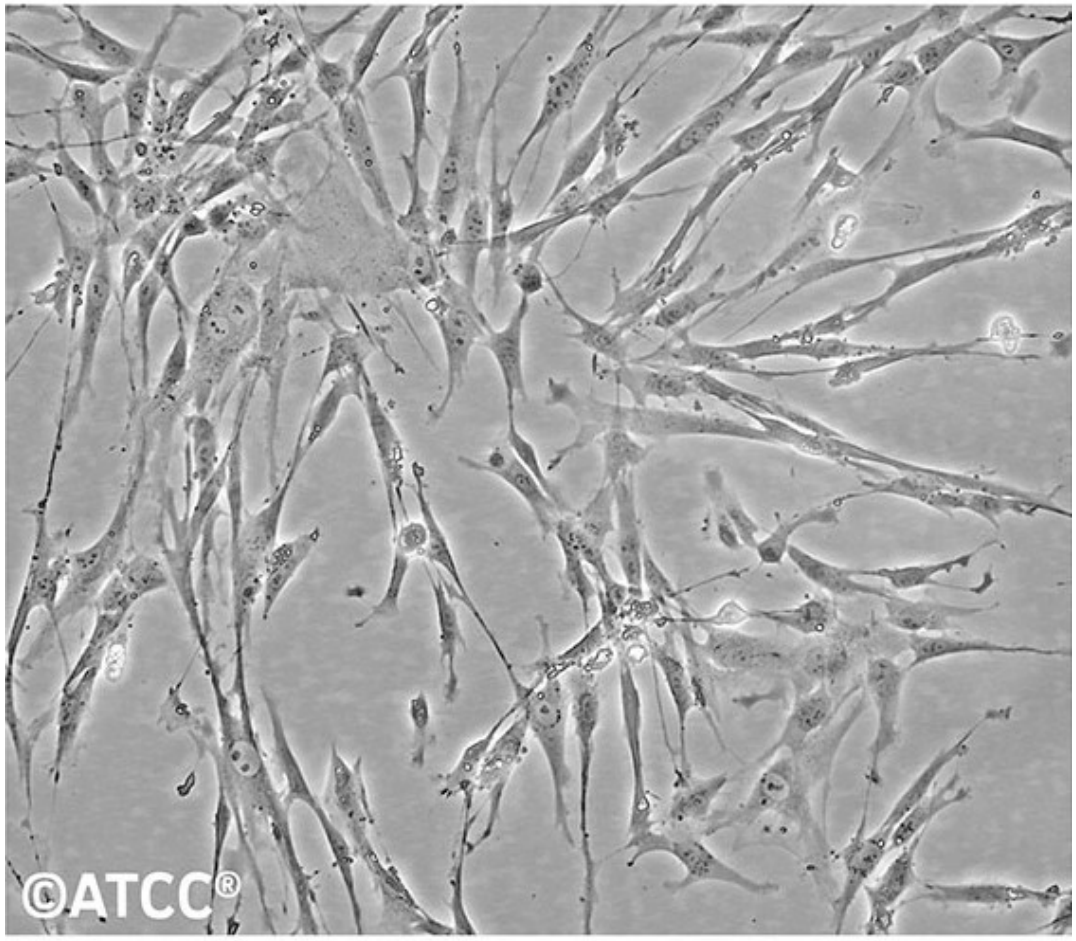



High Density

Scale Bar = 100µm

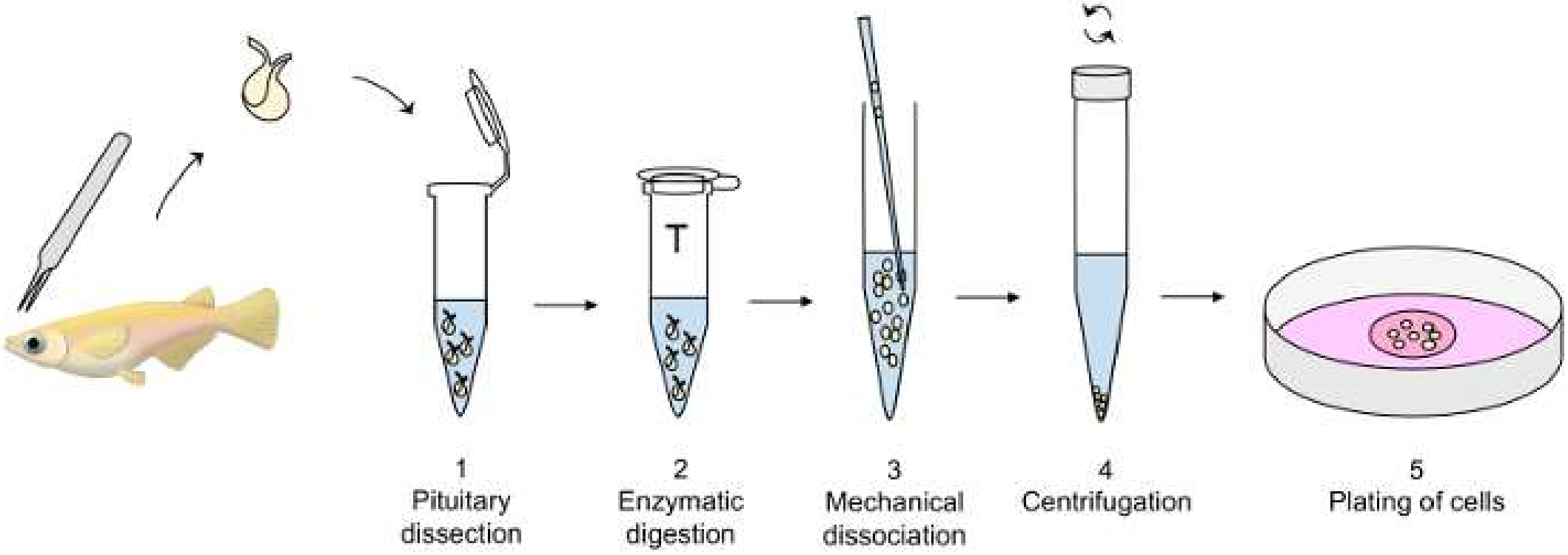
# Линия фибробластов легкого WI-38

Designation: **WI-38**

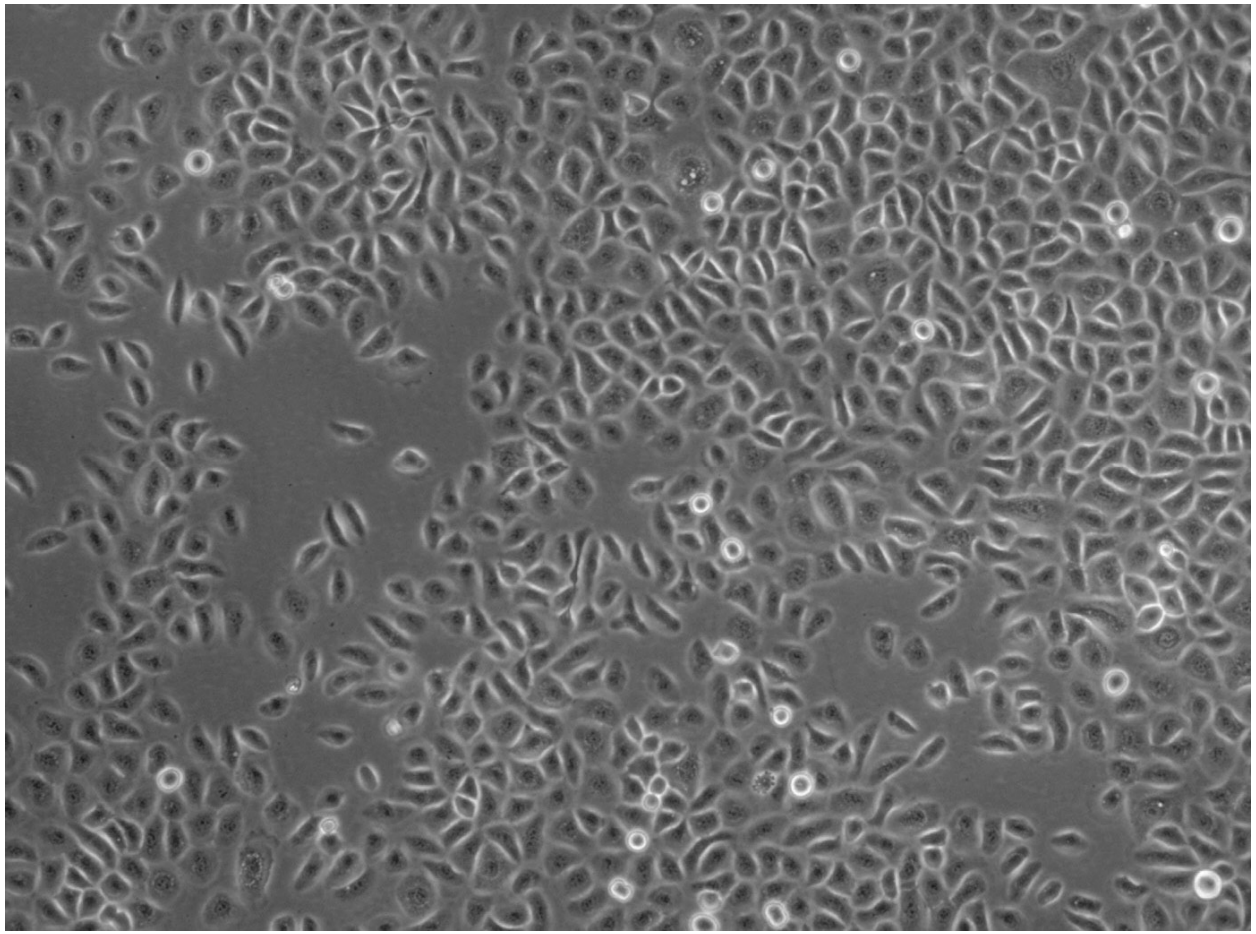



Low Density

# Выделение клеток из ткани

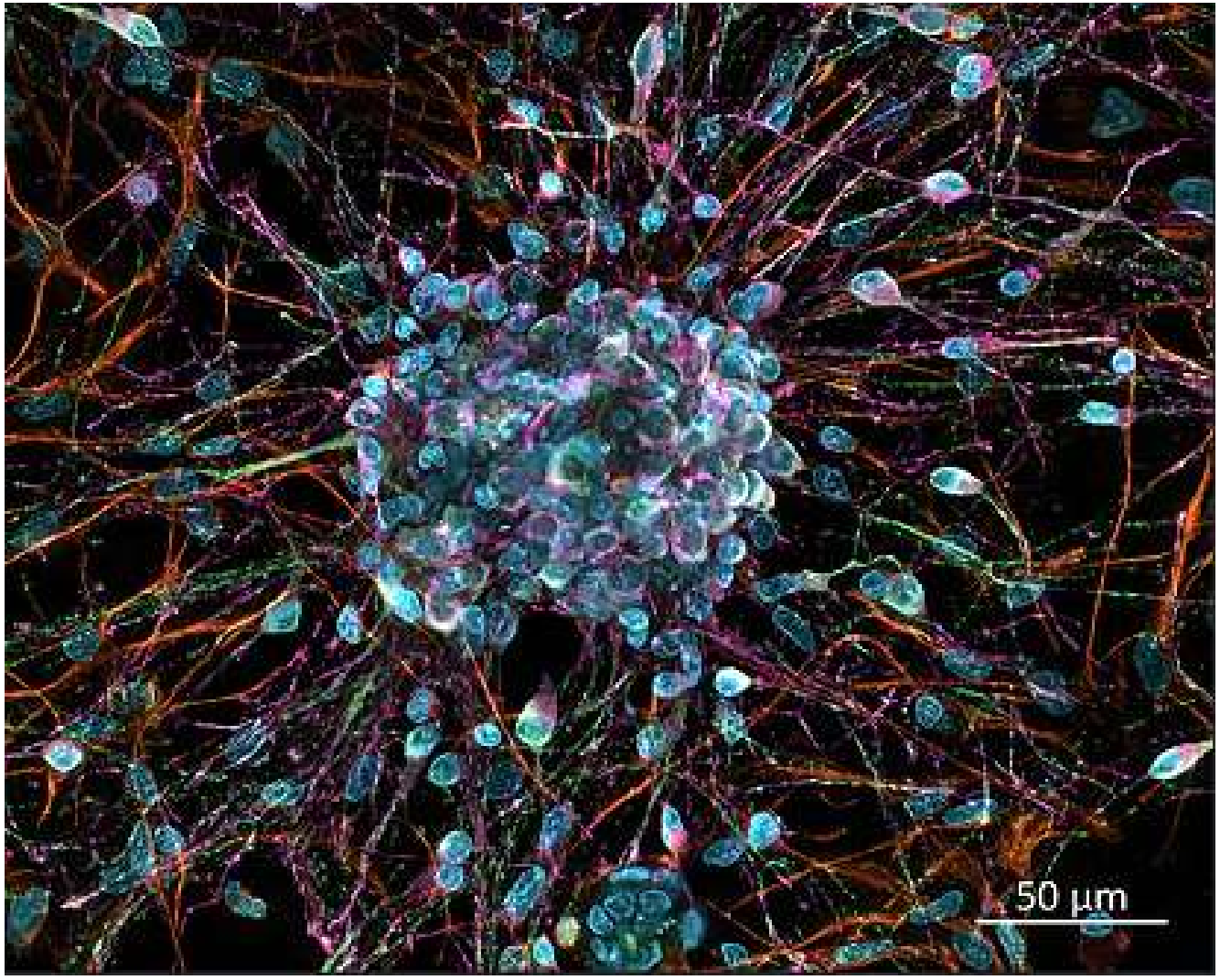


# Normal Human Epidermal Keratinocytes (NHEK)

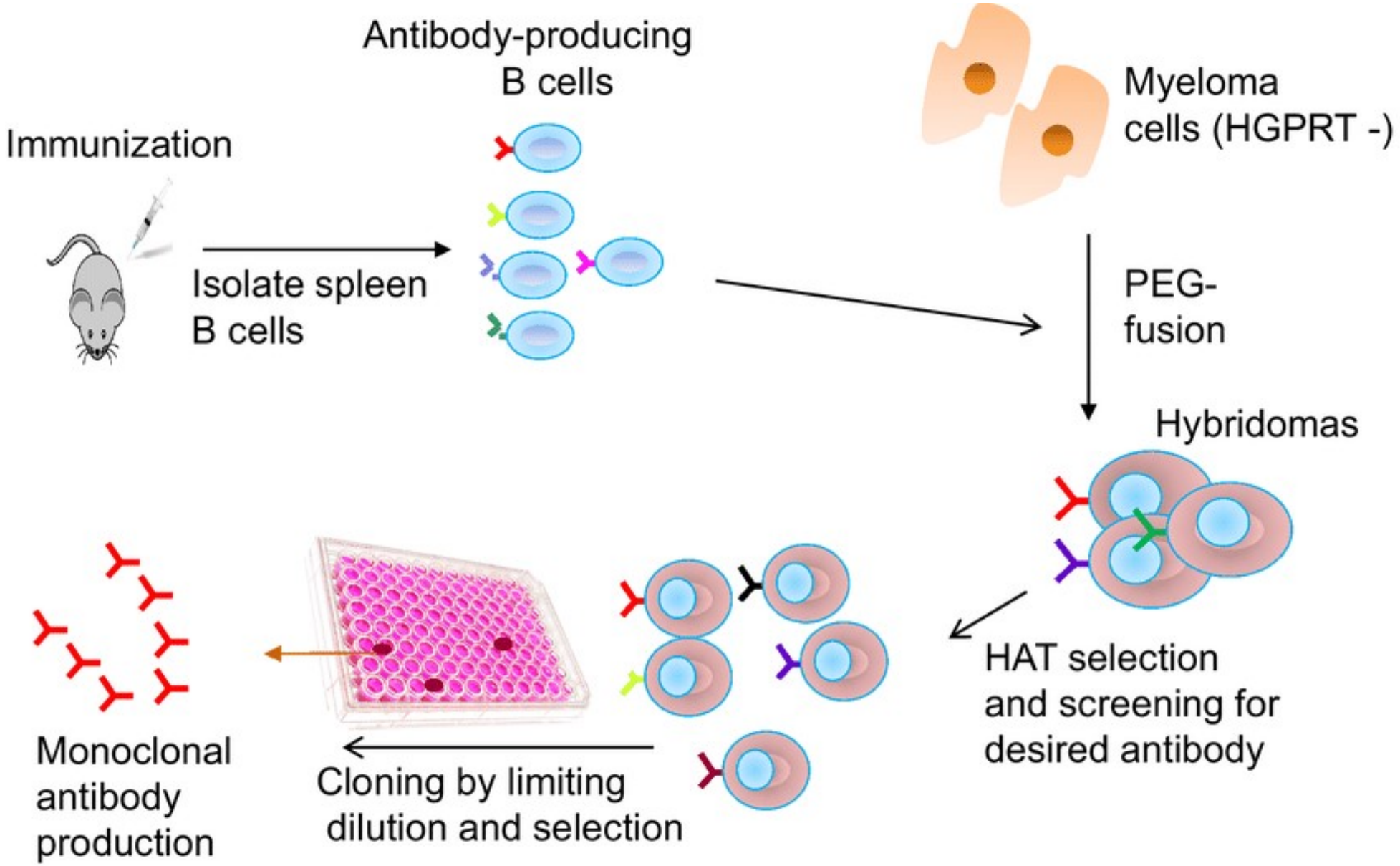


Primary Human Keratinocytes isolated from the epidermis of juvenile foreskin or adult skin from single or pooled donors

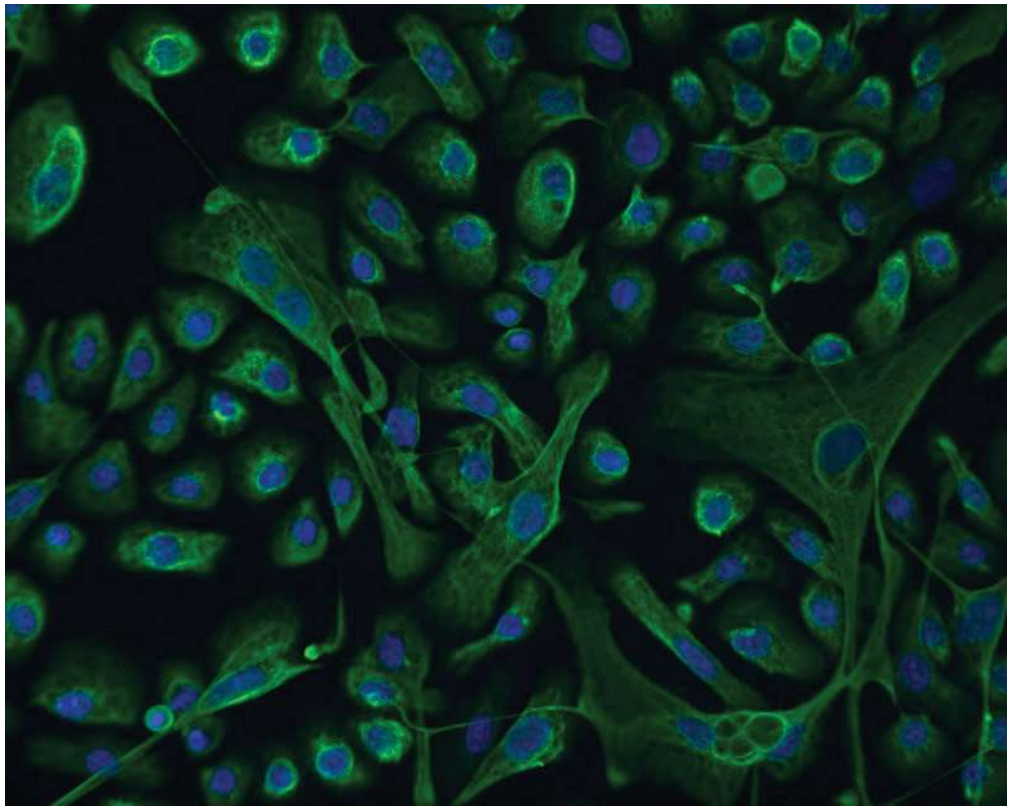
# Первичные культуры




нейроны


Antibody-producing
B cells
Myeloma
cells (HGPRT -)
Immunization
Isolate spleen
B cells
PEG-
fusion
Hybridomas
HAT selection
and screening for
desired antibody
Monoclonal
antibody
production
Cloning by limiting
dilution and selection

# ATCC CRL-4027 stained with a monoclonal pan-cytokeratin antibody (green) and Hoechst dye (blue)

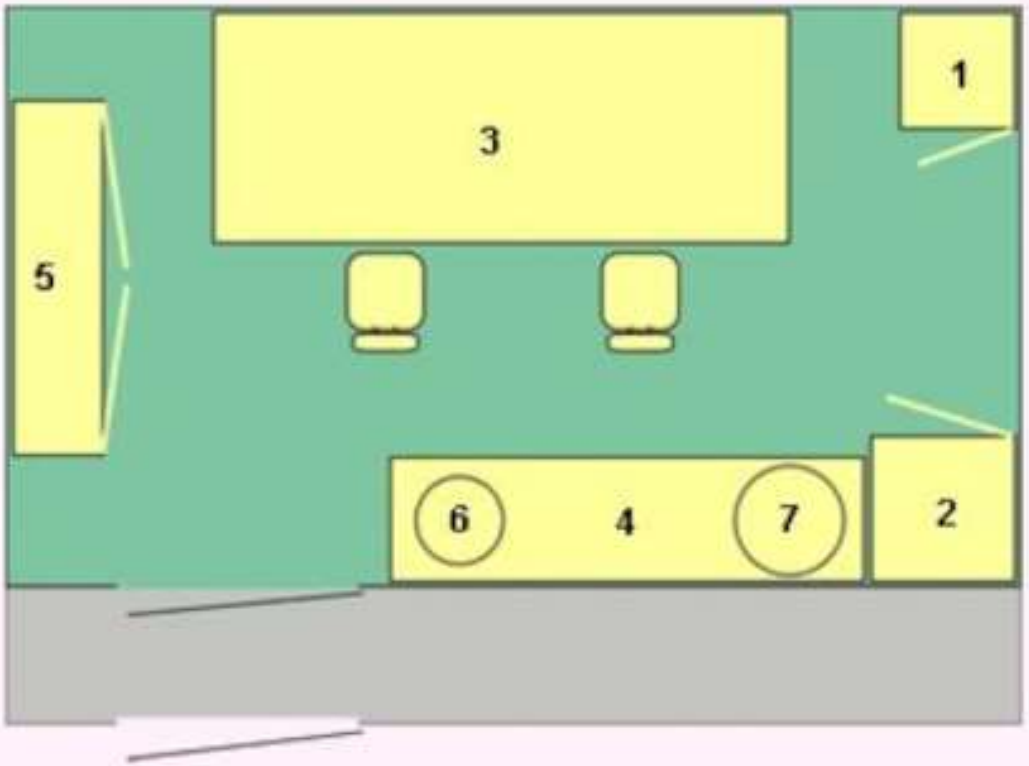


The Barrett's esophagus cell lines, CP-A (KR-42421), ATCC CRL-4027, CP-B (CP-52731), ATCC CRL-4028, CP-C (CP-94251), ATCC CRL-4029, and CP-D (CP-18821), ATCC CRL-4030 were derived from an endoscopic biopsy specimen obtained from a region of non-dysplastic metaplasia and transduced with the retroviral expression vector, pLXSN-hTERT.

# Коллекции клеток

- ATCC (American Type Culture Collection)
- Российская коллекция клеточных культур (РККК)
- European Collection of Authenticated Cell Cultures (ECACC)

# Культуральный блок




Помещение культурального блока:

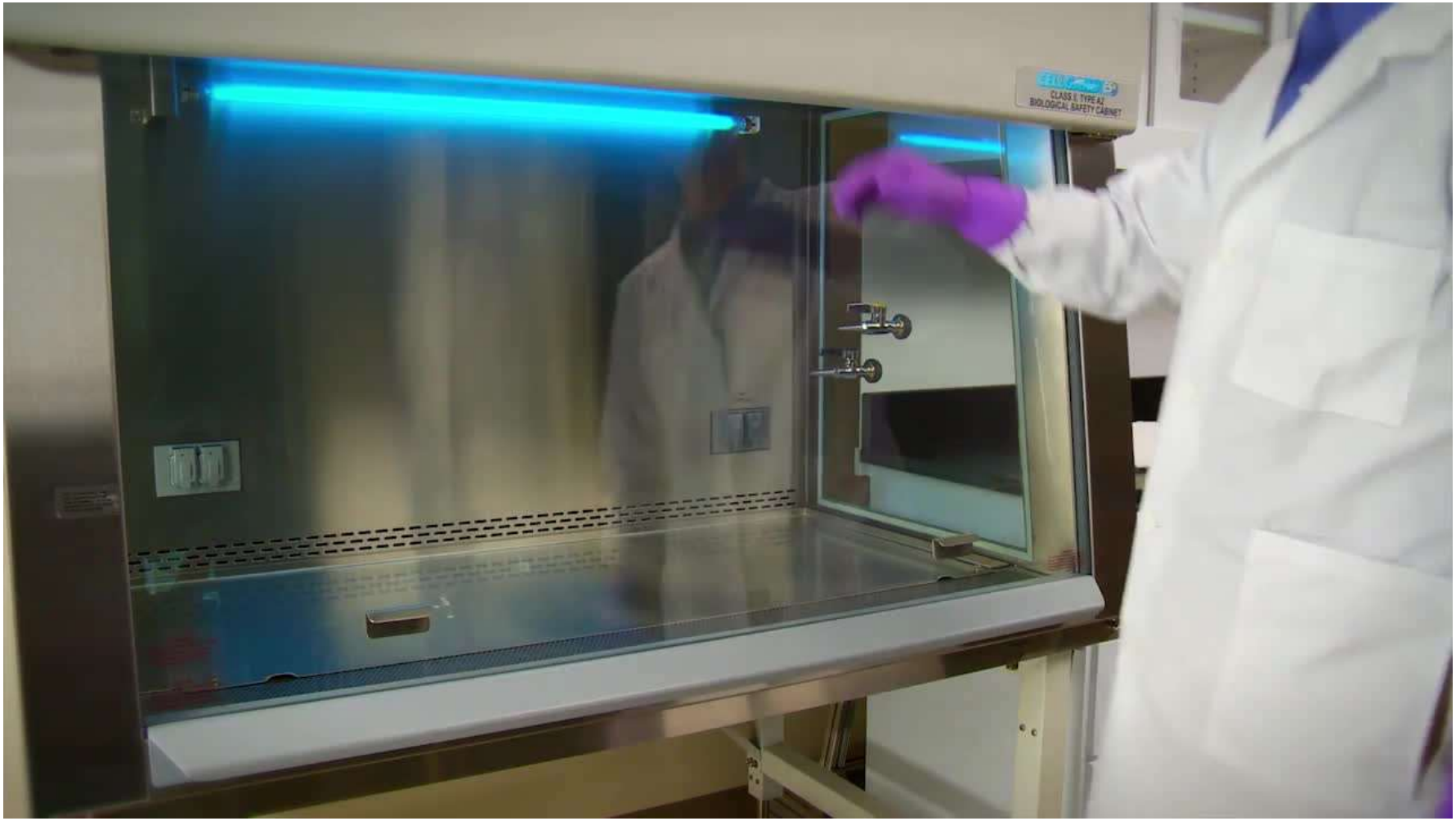
предбоксник (серый цвет)
и бокс (зеленый)

1 - $CO_2$-инкубатор
2 - холодильник
3 - ламинар
4 - стол с микроскопами:
световым (6)
и инвертированным
световым (7)
5 - шкаф с посудой

# Ламинарный шкаф


ВЫХОД ОТФИЛЬТРОВАННОГО ВОЗДУХА
HEPA Filter
HEPA Filter
HEPA - Высокоэффективная задержка частиц
ПОСТУПЛЕНИЯ КОМНАТНОГО ВОЗДУХА ЧЕРЕЗ СТВОРКУ
ЗАГРЯЗНЕННЫЙ ВОЗДУХ
Воздух, прошедший через HERA фильтр
КОМНАТНЫЙ ВОЗДУХ
Вентилятор

# УФ-лампы



# $CO_2$-инкубатор

# Культуральная посуда

Флакон культуральный с вентилируемой крышкой


Пробирка культуральная со скошенным дном

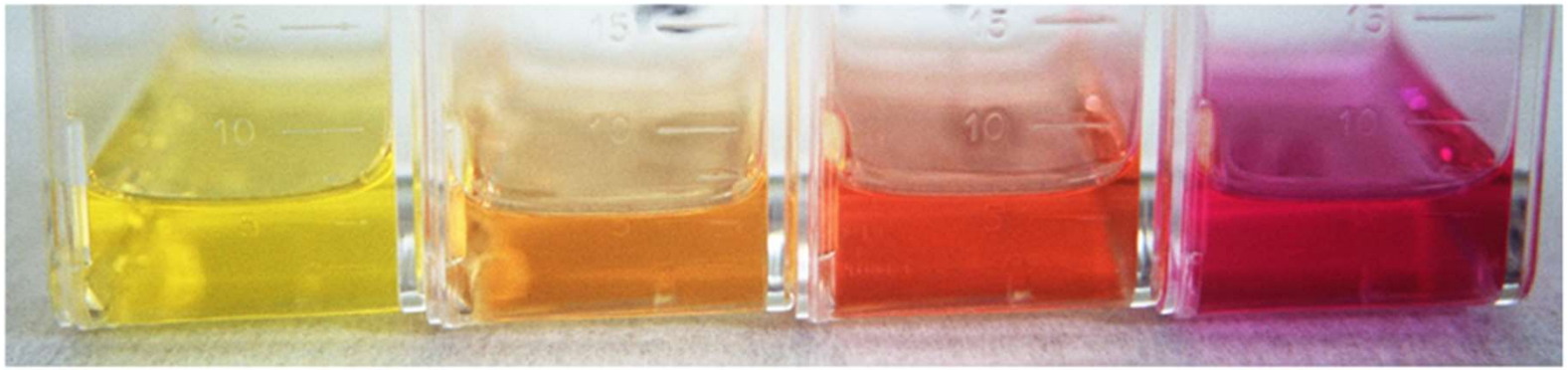
Планшет культуральный

Чашка Петри культуральная

# Среды для культивирования клеток

# Среды для культивирования клеток

| Change today / check for contamination | Change within 24-48h | Leave | Change or re-gas with $CO_2$ / check incubator / $CO_2$ supply |
|---|---|---|---|

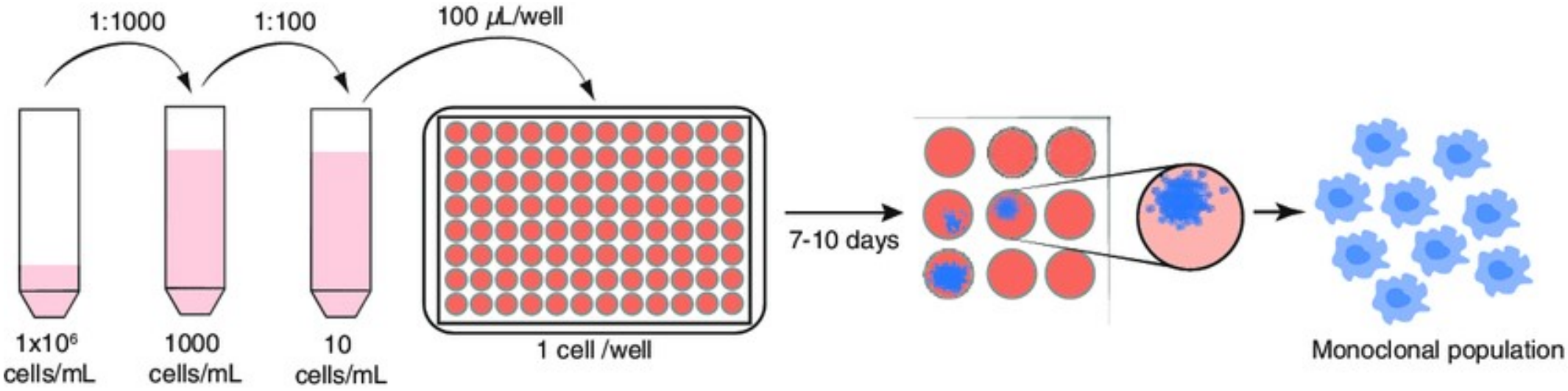


| pH | 6.5 | 7.0 | 7.4 | 7.8 |
|---|---|---|---|---|

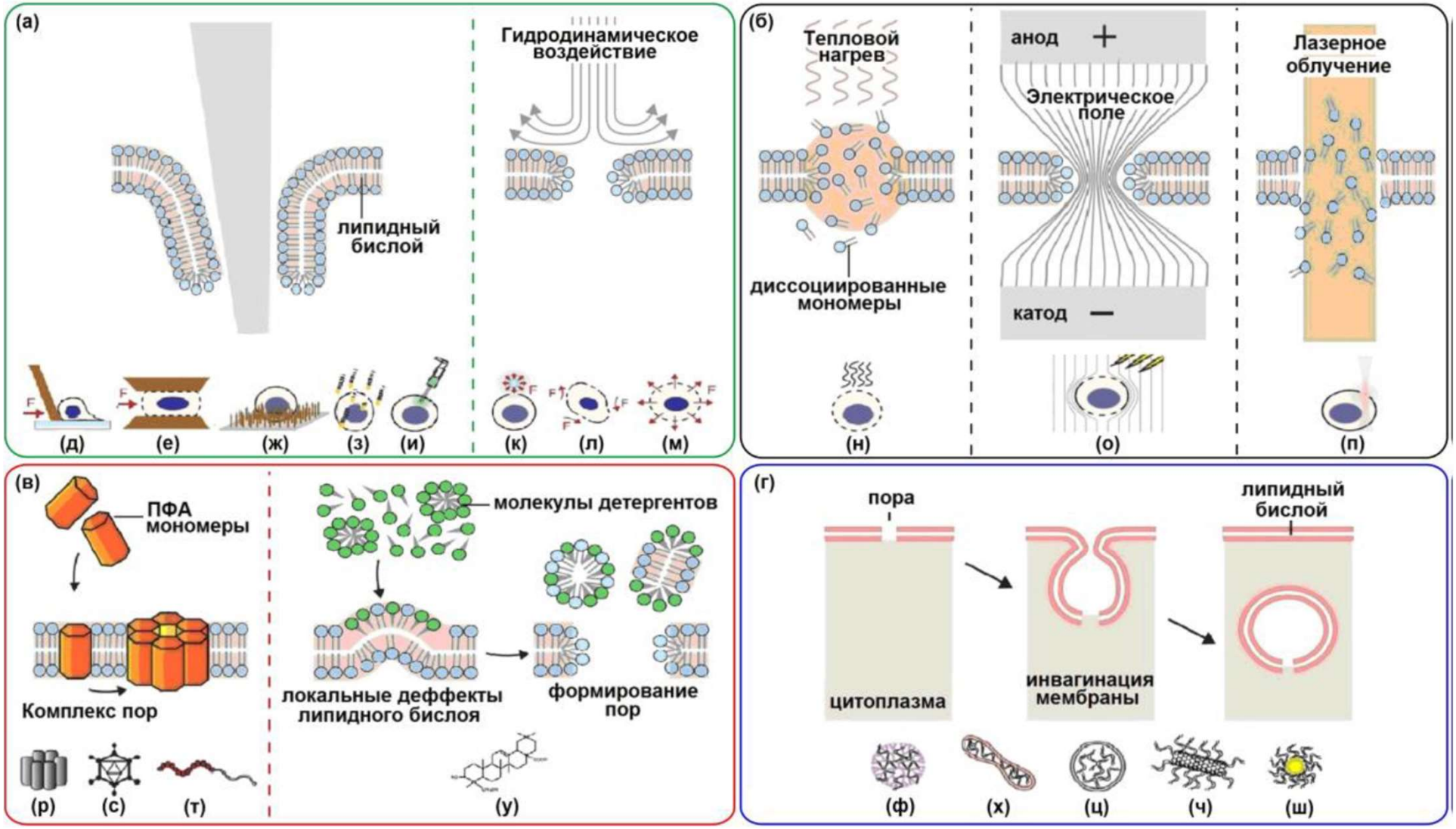
# Дозаторы


# Криоконсервация клеток

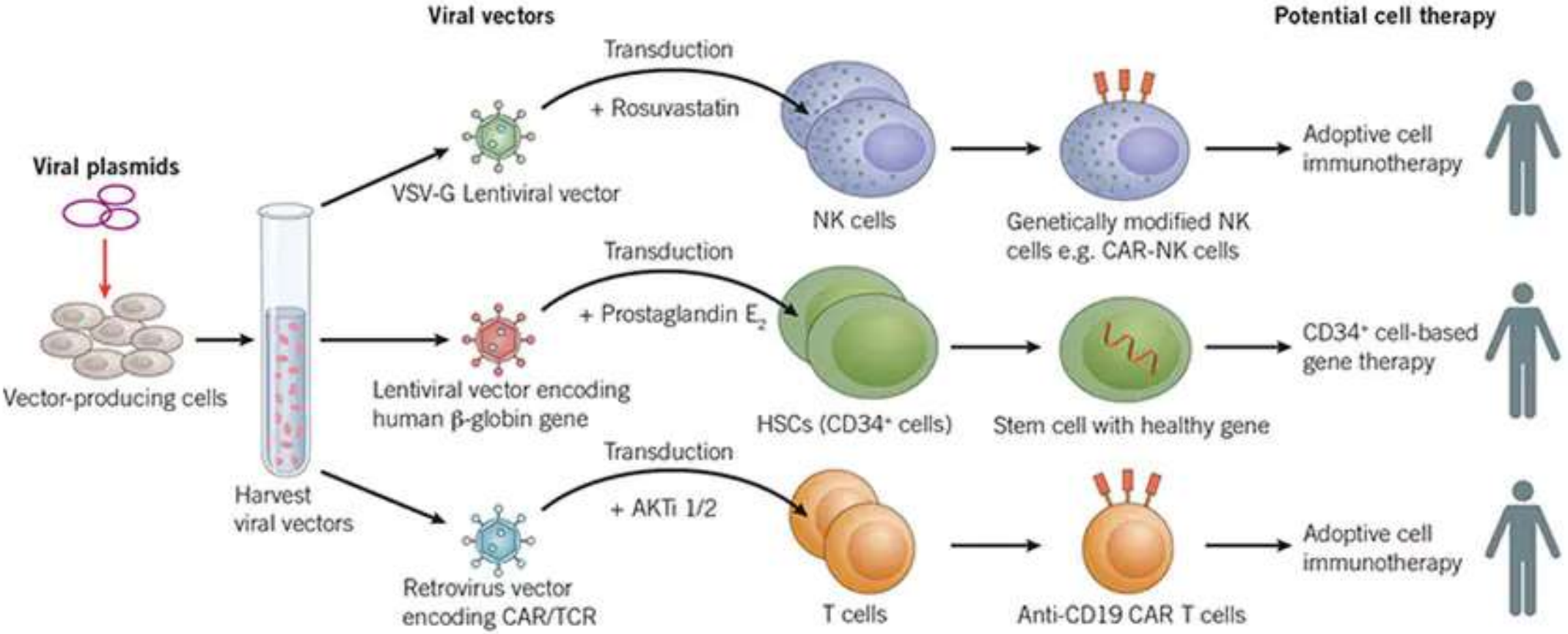
# Клонирование клеток



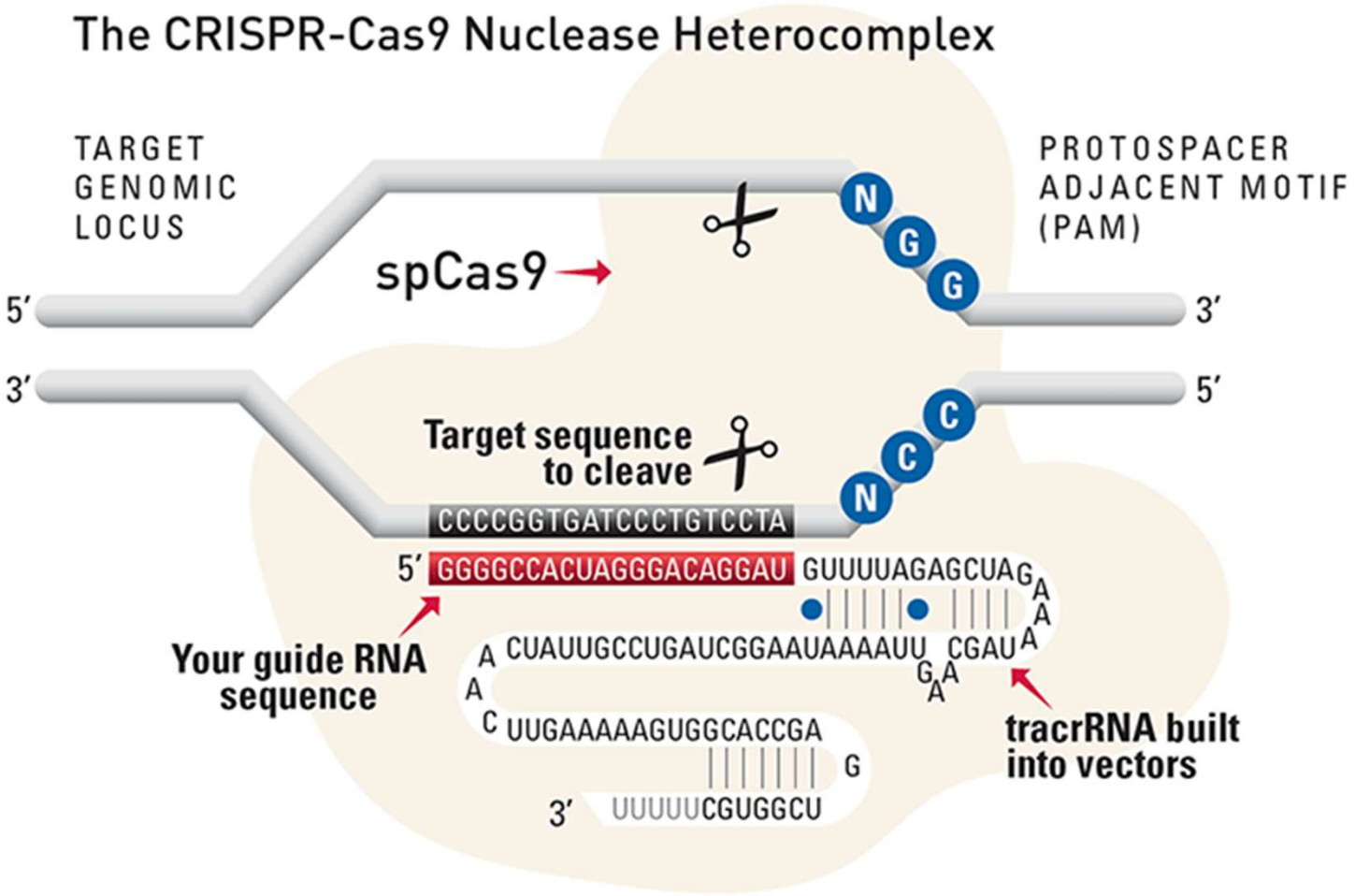
# Доставка генов в клетки

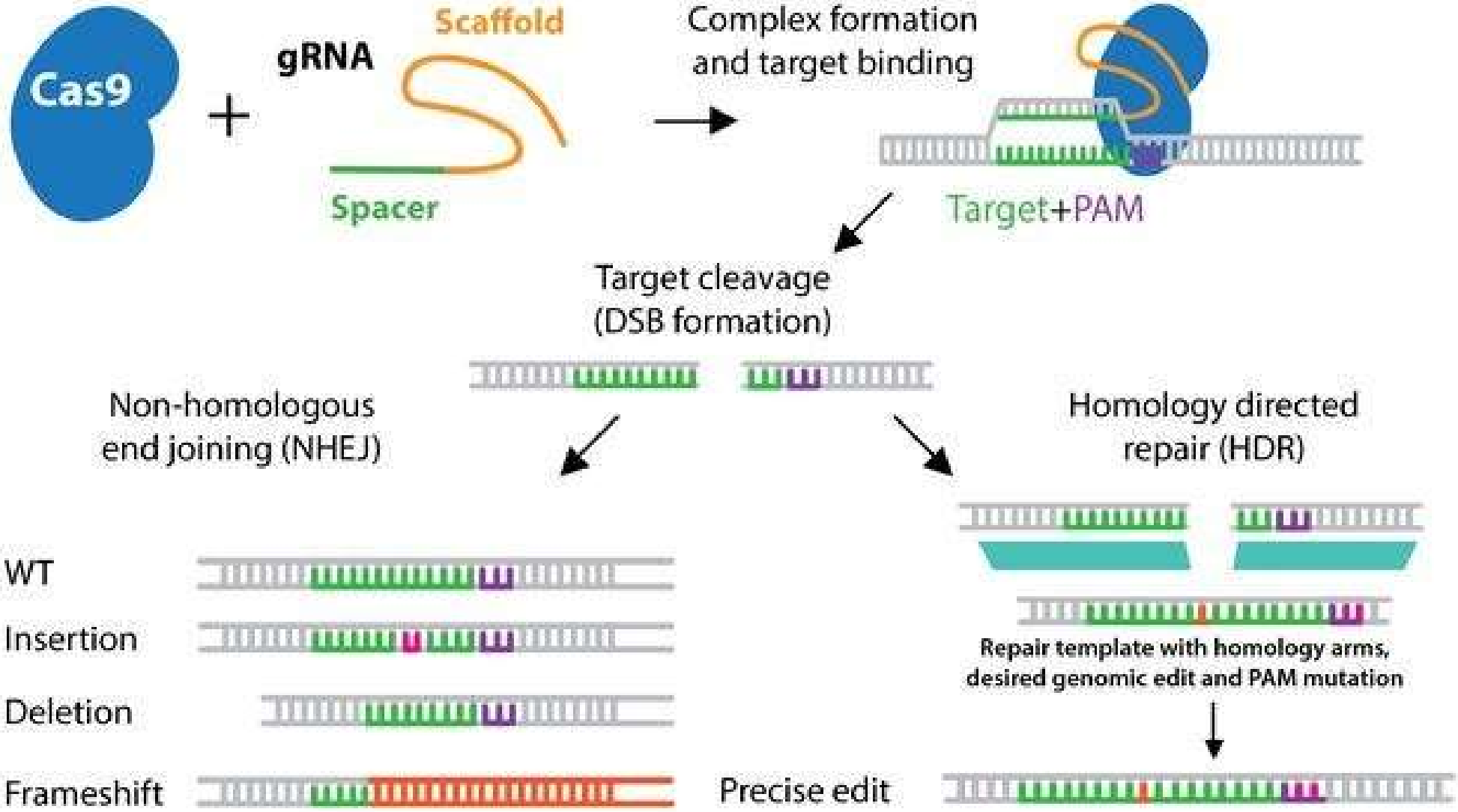


# Вирусная трансдукция

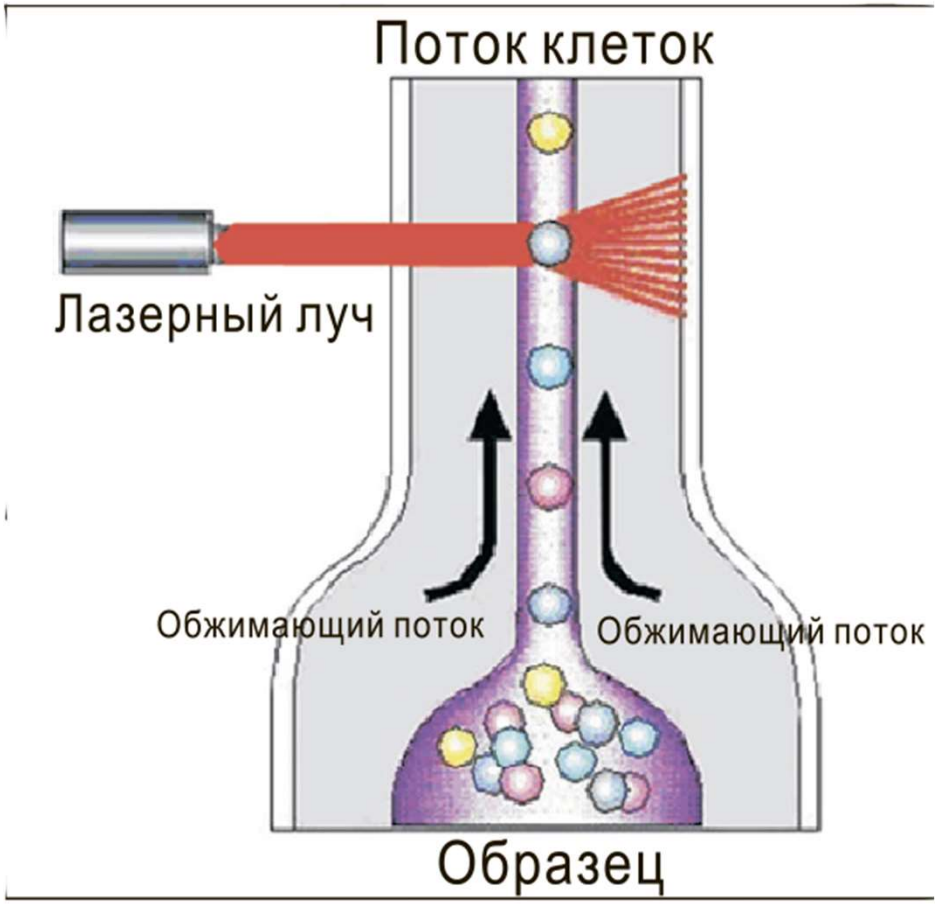


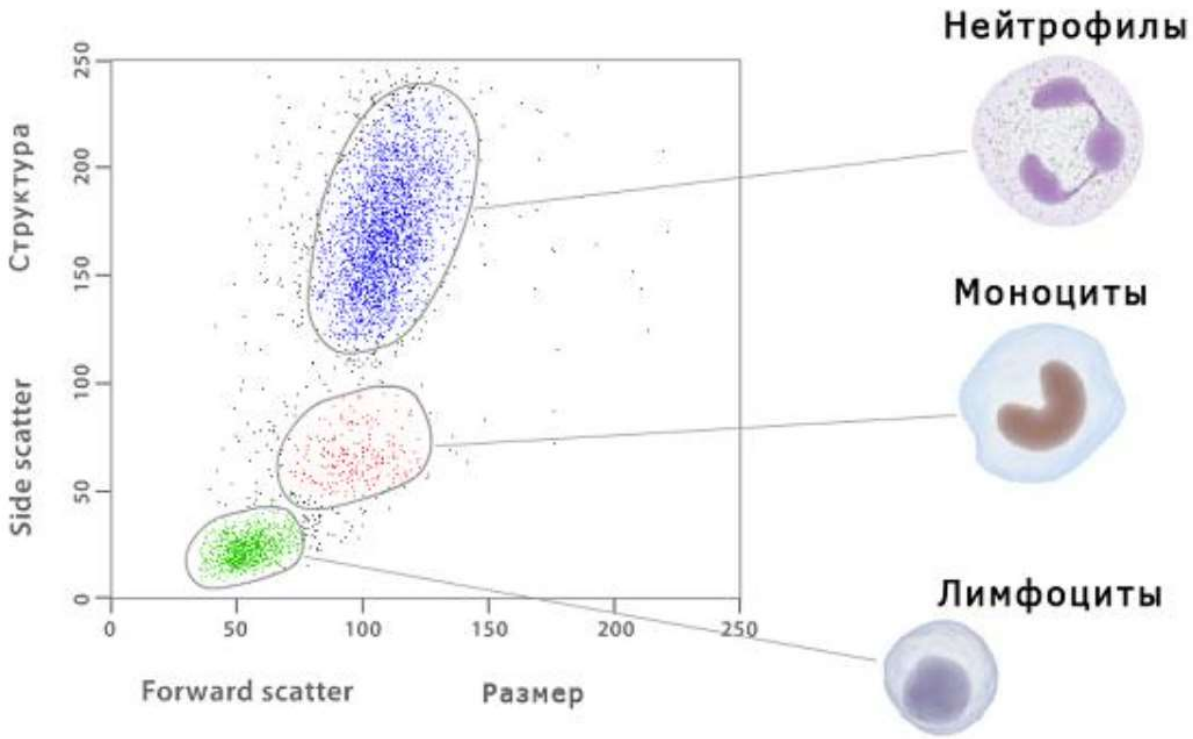
# CRISPR/Cas-9


The CRISPR-Cas9 Nuclease Heterocomplex
TARGET GENOMIC LOCUS
PROTOSPACER ADJACENT MOTIF (PAM)
spCas9
5′
3′
3′
5′
N
G
G
C
C
N
Target sequence to cleave
CCCCGGTGATCCCTGTCCTA
5′ GGGGCCACUAGGGACAGGAU GUUUUAGAGCUA
A CUAUUGCCUGAUCGGAAUAAAAUU CGAU
C UUGAAAAAGUGGCACCGA
G
3′ UUUUUCGUGGCU
Your guide RNA sequence
tracrRNA built into vectors


Cas9
gRNA
Scaffold
Spacer
Complex formation
and target binding
Target+PAM
Target cleavage
(DSB formation)
Non-homologous
end joining (NHEJ)
Homology directed
repair (HDR)
WT
Insertion
Deletion
Frameshift
Repair template with homology arms,
desired genomic edit and PAM mutation
Precise edit

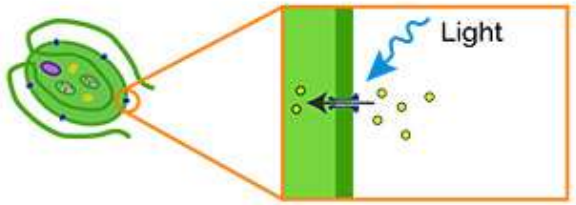
# Проточная цитометрия



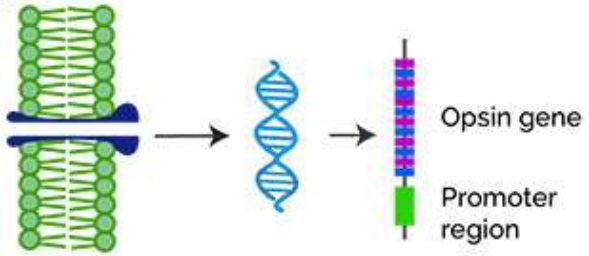
# Оптогенетика

1



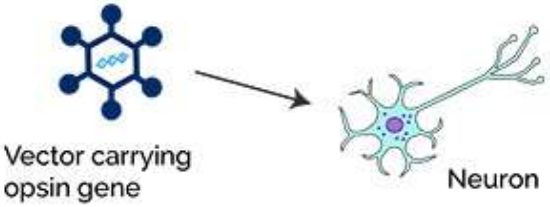

Light sensitive opsin proteins such channelrhodopsin-2, are found in algae and bacteria. They are transmembrane proteins that act as ion channels and can be activated by specific light wavelengths, in this case blue light.

2



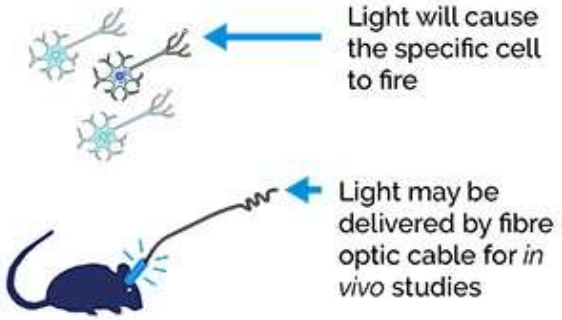

The opsin gene sequence can be extracted from the donor organism so they may be inserted into other cells to serve as a research model.

3



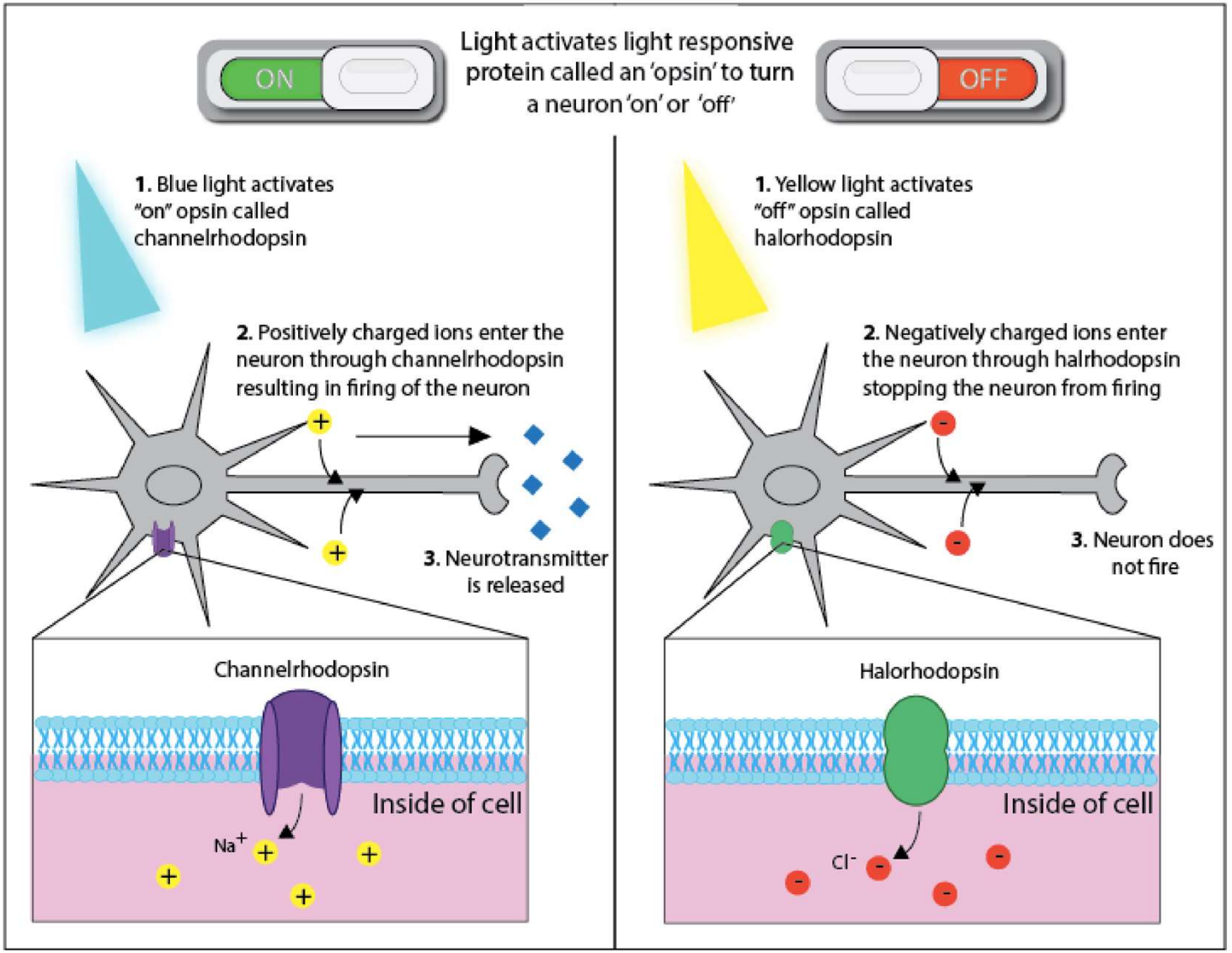

The opsin gene sequence can be introduced into specific cells - for example using a virus vector to insert the gene into a neuron.

4




Light can thus be used with millisecond precision, to activate only the specific cells of interest, resulting in neuronal firing and the effects can be determined. This degree of precision could not be done using conventional electrical or chemical means.

# How does optogenetics work?

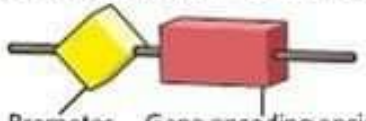


# SIX STEPS TO OPTOGENETICS

With optogenetic techniques, researchers can modulate the activity of targeted neurons using light.

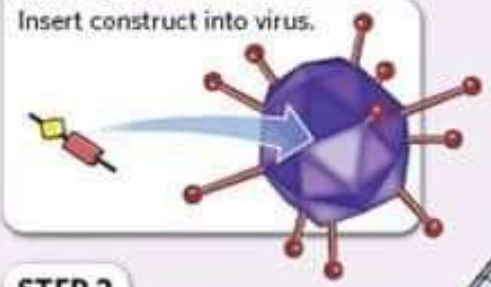
**STEP 1**

Piece together genetic construct.



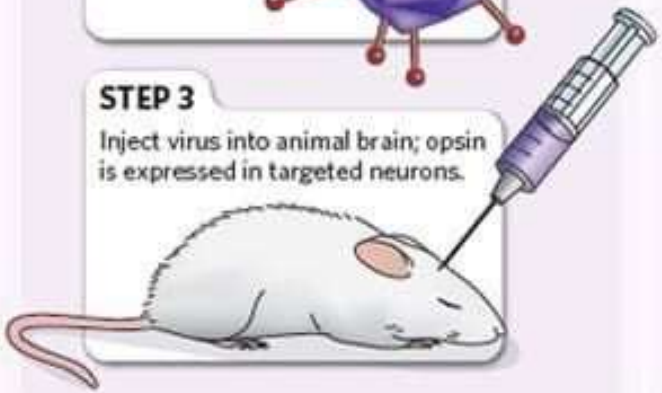

**STEP 2**

Insert construct into virus.



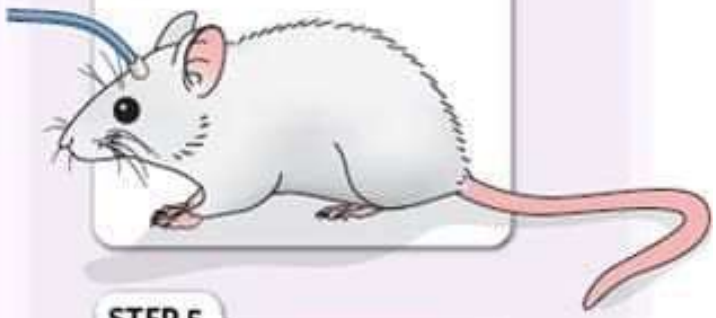
**STEP 3**

Inject virus into animal brain; opsin is expressed in targeted neurons.



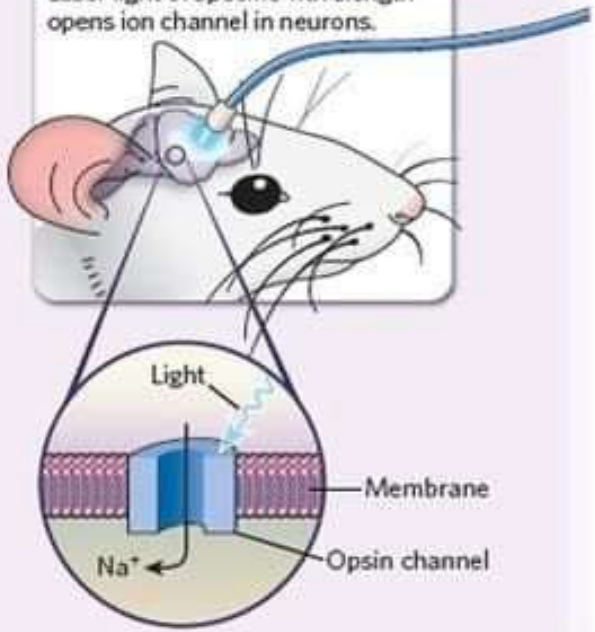
**STEP 4**

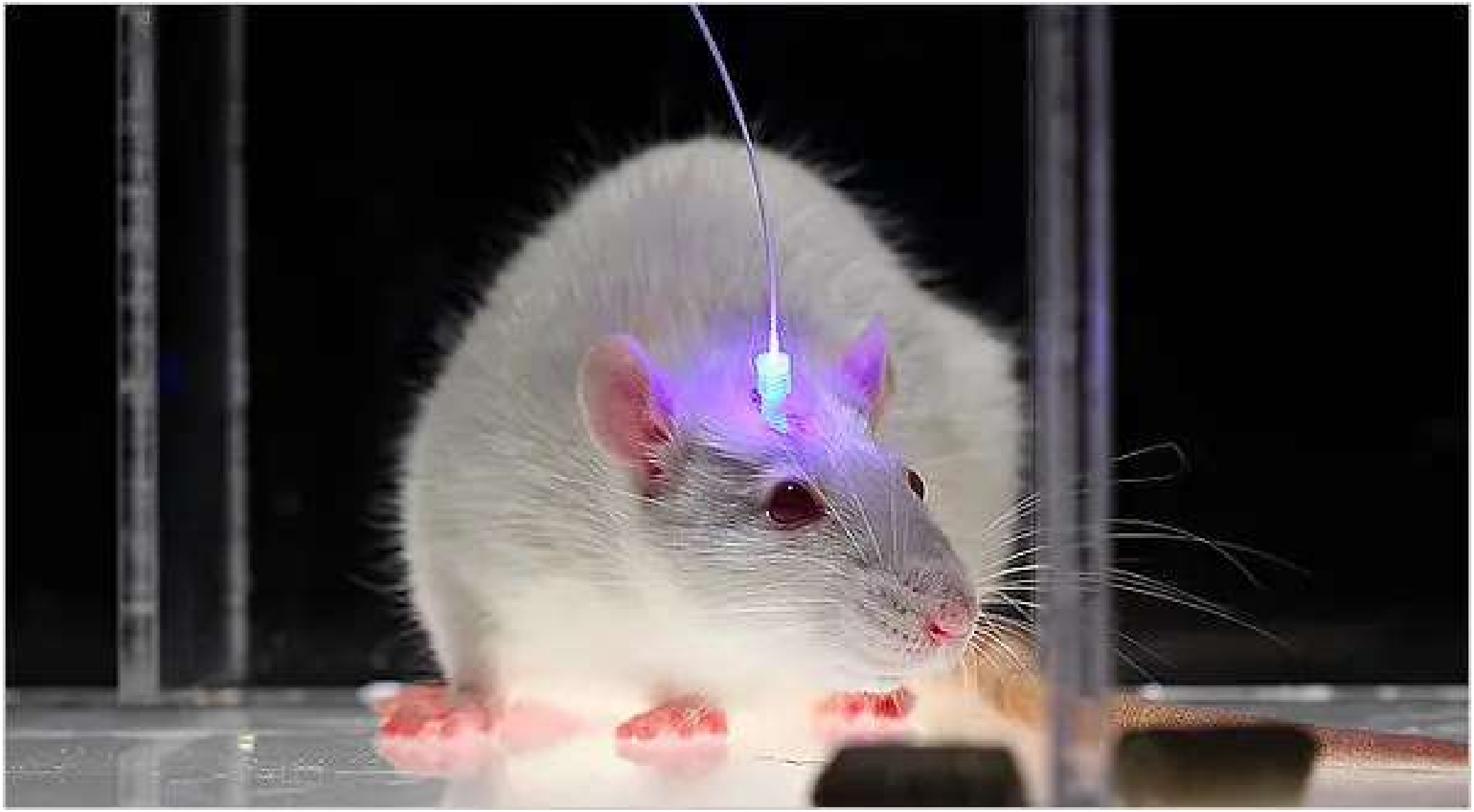
Insert 'optrode', fibre-optic cable plus electrode.



**STEP 5**

Laser light of specific wavelength opens ion channel in neurons.






# Благодарю за внимание!