PRODUCTION OF VIRUSES AND HARVESTING

Khder Hussein Rasul

Zahra Mohammad Maghded

Practical Medical Virology and MA 403

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Outline

- Production of viruses
- Harvesting of viruses

Objectives

- Get knowledge about techniques of virus production
- Learn about cell culture transfection and steps
- Get information about harvesting of produced viruses

Viral production and techniques

- **Viral production** refers to the process of generating a significant quantity of viruses for various purposes, such as scientific research, vaccine development, or gene therapy.

- **Common techniques for virus production**

1. Cell culture
2. Embryonated egg
3. Animals (in vivo)
4. Bacterial systems
5. Plant systems

Cell culture transfection

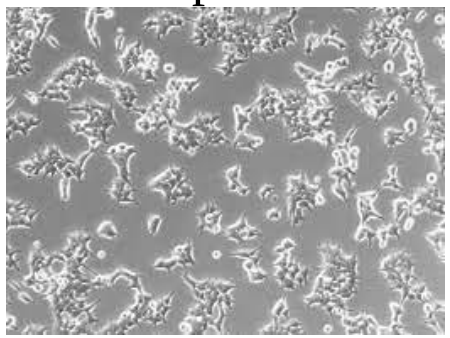
- **Cell culture transfection** is a common method used in molecular biology and virology to produce viruses, particularly viral vectors for research.
- This process involves introducing viral genetic material (DNA or RNA) into cultured host cells, allowing the cells to express and produce the virus or viral vector.

- General steps for using cell culture transfection to make viruses are

1. **Select host cells:**

Choose the appropriate host cells that are permissive for the virus you intend to produce. Different viruses may require specific cell lines for optimal replication.

Example: **Lentivirus is produced in HEK293FT cells.**



Cell culture transfection

2. Prepare cells for transfection:

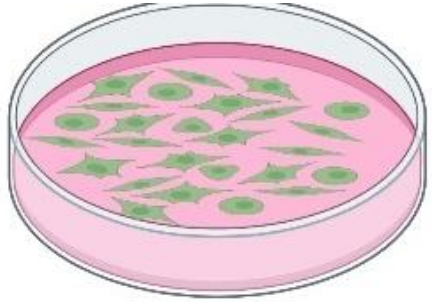
To reach a high yield of lentiviral particles per ml, it is crucial to consider the following points

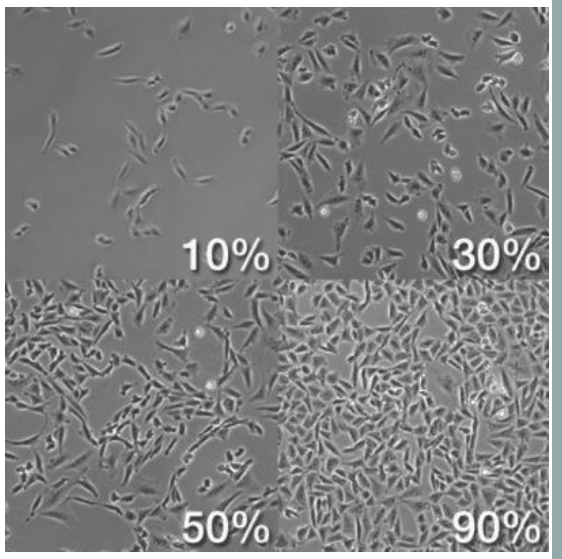
A. Use HEK293FT cells that are in the exponential growth curve.

B. Make sure HEK293FT cells never reach **full confluency** before virus production. Roughly, a confluency of **60-80%** is ideal.

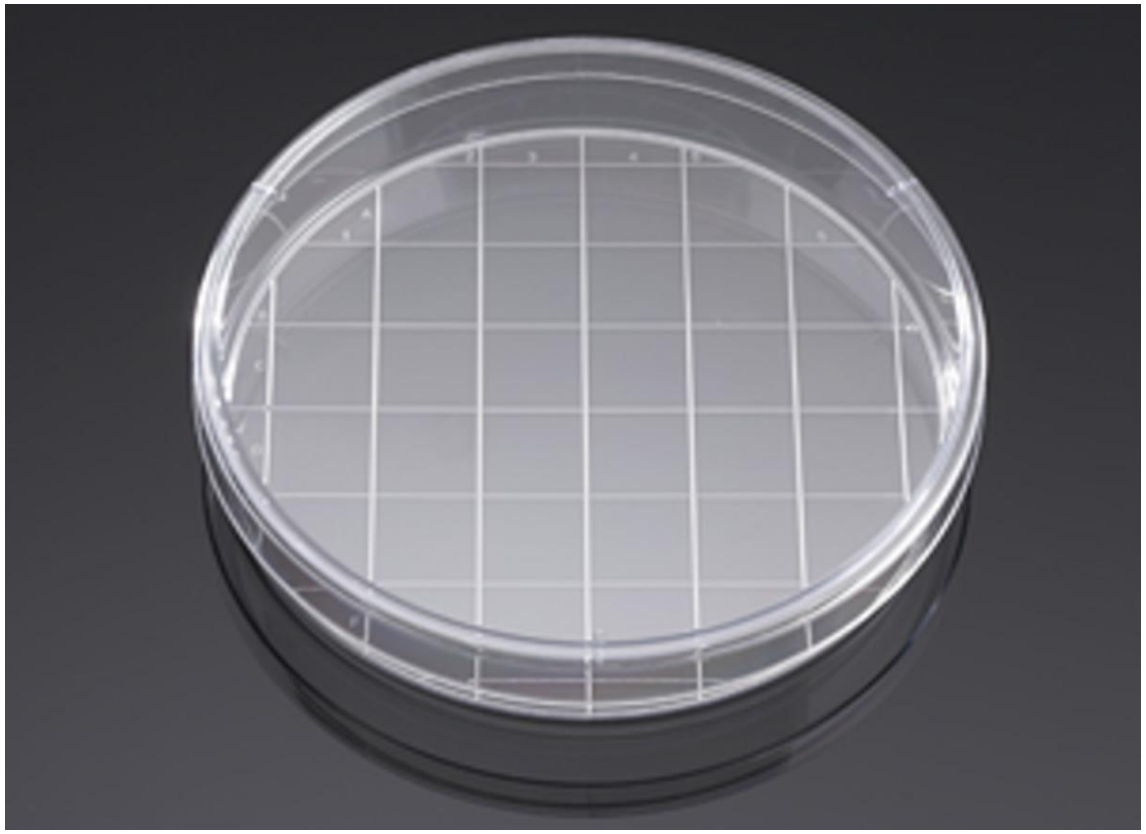
C. Use HEK293FT cells between passage number **3-20.** Never use HEK293FT cells beyond passage **20**.

Apply cell suspension onto a 150 mm poly-D-lysine coated cell culture plate and place in incubator for 8 hours.





Cell culture transfection

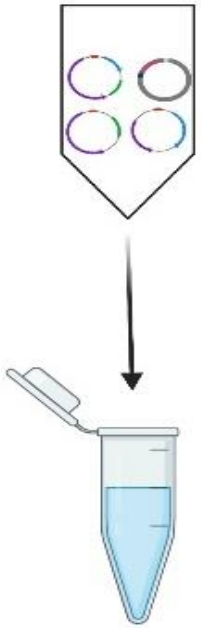


Cell culture transfection

3. Prepare viral genetic material:

Prepare plasmids or viral DNA containing the genes necessary for virus replication and assembly. This may include **the viral genome**, **essential structural genes**, and **any desired modifications**.

1. VSV-G
2. REV
3. Gag pol



- VSV-G is envelope plasmid to enhance the tropism and increase stability.
- Rev is essential for the proper export of unspliced or partially spliced viral RNA from the nucleus to the cytoplasm.
- Gag pol contains genetic information for the expression of key viral proteins necessary for the assembly.

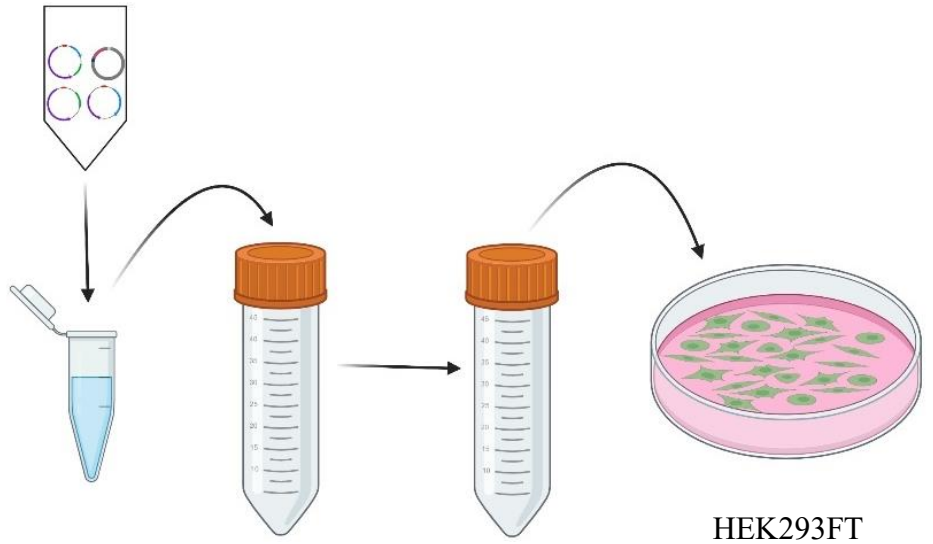
1. Gag encodes for structural proteins that are responsible for the formation of the capsid.
2. Pol encodes for viral enzymes, primarily reverse transcriptase, protease, and integrase.

Cell culture transfection

4. Transfection:

- Use a suitable transfection method to introduce the viral genetic material (plasmids) into the host cells.
- In transfection step, plasmids are added into seeded cells

VSV-G
REV
Gag pol



- Common transfection methods include **chemical transfection (e.g., calcium phosphate, lipofection)** or **physical transfection (electroporation).**

Cell culture transfection

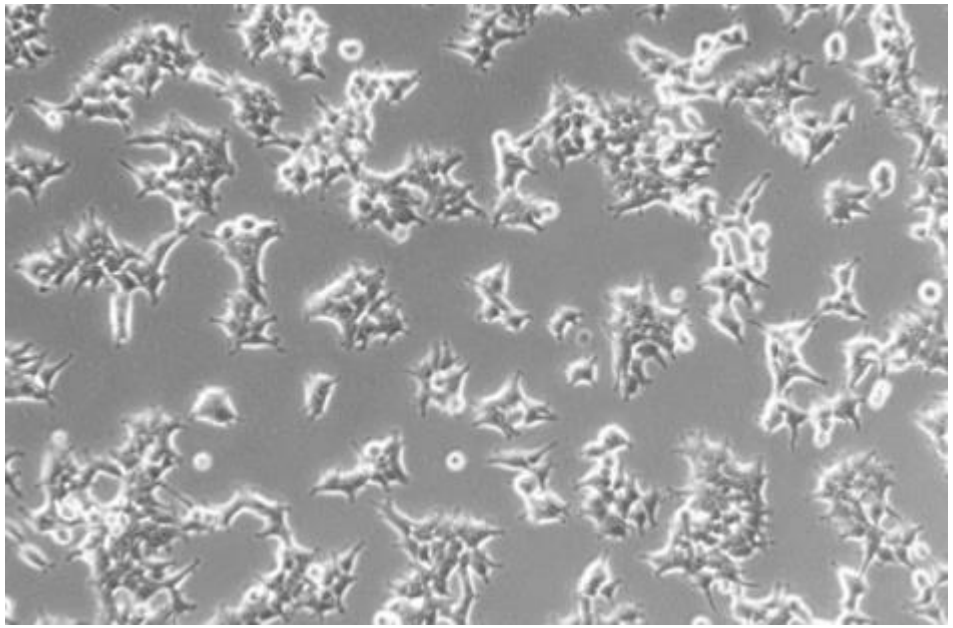
5. Incubation:

- Allow the transfected cells to incubate under conditions that promote virus replication and assembly. This may include maintaining appropriate temperature, humidity, and nutrient levels.
- Incubate the plate at 37°C, 5% CO2 for 12-16 hours.

Cell culture transfection

Day 1: Medium change

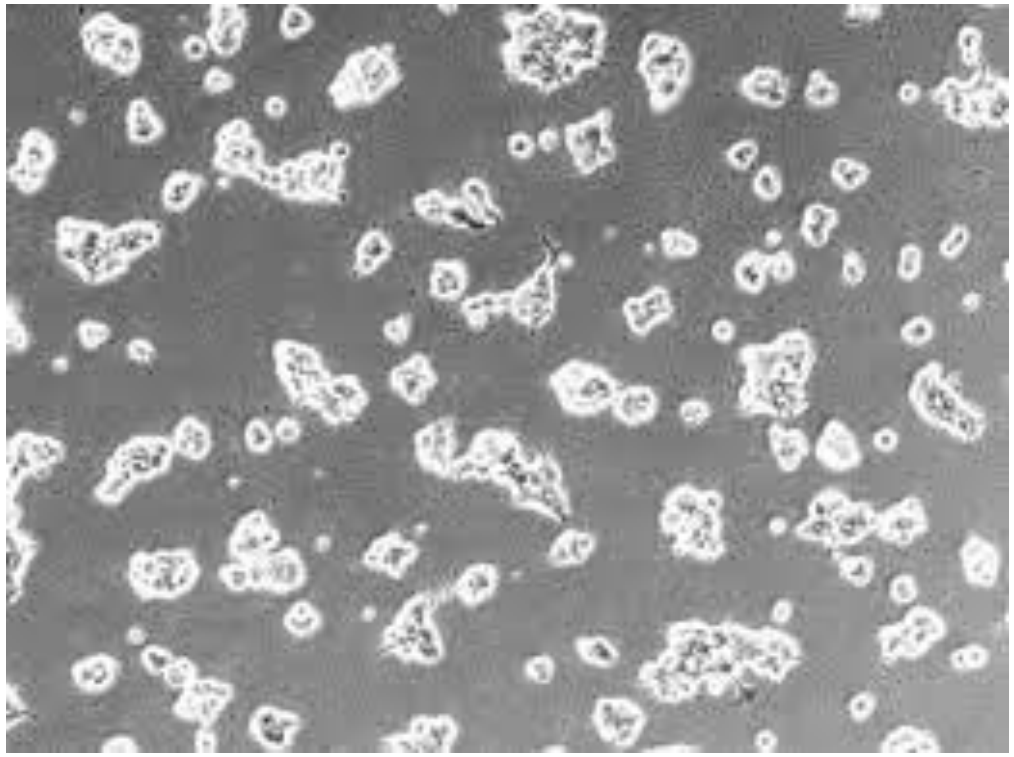
1. Analyze HEK293FT cells under light microscope. Check for a good confluency (70 - 80 %) and signs of infection (rounded edges of the cells)
2. Remove medium carefully from the plate and replace it with **prewarmed full growth medium**.
3. Place back the plate into incubator.



Cell culture transfection

6. Virus Production:

As the transfected cells express and replicate the viral genetic material, new viral particles are generated. These particles can be released into the cell culture medium.



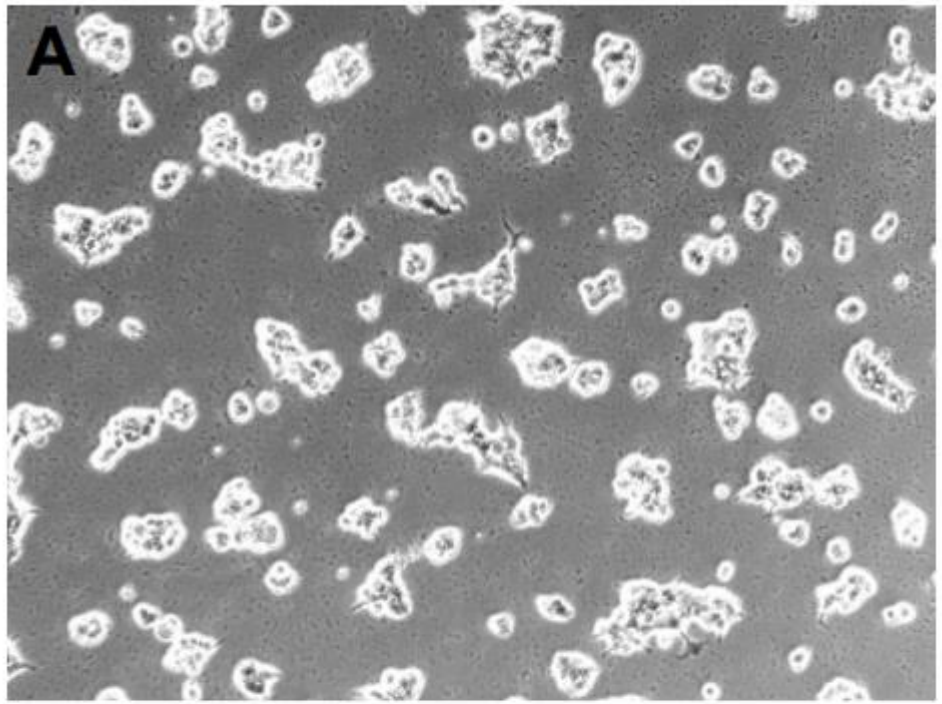
Cell culture transfection

7. Day 2: Collection of 24 hr supernatant

- Analyse HEK293FT cells under light microscope. At this stage you should see clear signs of infections as described for the medium change and also some cells will have **detached** from the cell culture dish.
- Harvest the medium from the plate, using a 20 ml syringe. Carefully tilt the plat and suck up the medium with the syringe. Avoid getting air into the syringe as contact with air will reduce the viral titer.
- Apply the 0.45 um filter onto the syringe and filter the supernatant against the inner wall of a 50 ml falcon tube. Avoid air bubbles and extreme force.

Cell culture transfection

Cell culture transfection



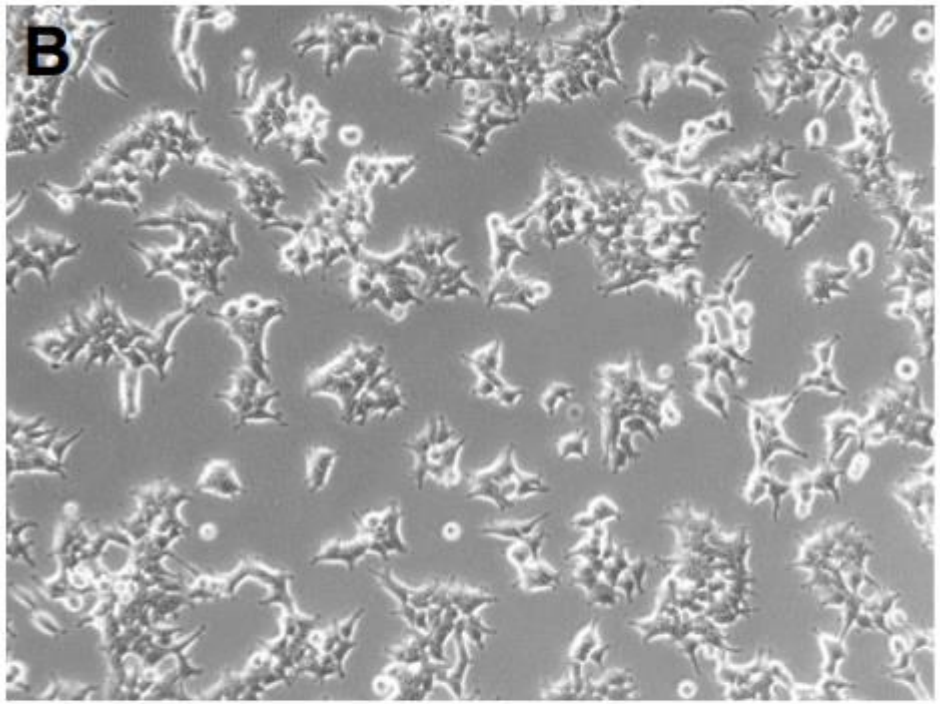


Fig. 1. A. 293T cells one day after transfection with DNA (10X phase contrast image). Cells are more contracted and less well spread than non-transfected cells, and small black particles ($CaHPO_4$/DNA precipitates) can be seen scattered across the plate in the spaces between cell clusters. **B.** Non-transfected 293T cells.

References (in APA style)

- Z. Debyser, Biosafety of lentiviral vectors. Curr Gene Ther 3 (2003) 517-25.
- Wang, X. and M. McManus (2009). "Lentivirus production." J Vis Exp (32).