**Working with cells**

2 types of cell cultures:

* 1. Adherent cells (the ones we use)
  2. Suspended cells
* The cells are grown in a special petri dish (we use 96)
* The cells are grown in a specific medium (contains mainly proteins), specific to the cell type. The most common medium is DMEM - it is commercial, and into to it you add serum (5-10%) and antibiotics (penstrep), sometimes also antifungal (nestrin).
* When the medium is added to the cells, it has to be at ~37⁰C.
* All work is performed in the hood:
  1. The hood has to be opened half way, ideally turned on 15 minutes before starting.
  2. Anything going into the hood is sprayed with ethanol 70%.

**Splitting a cell plate:**

When cells are covering most of the plate's surface it is needed to split the plate into several plates:

* Prepare ahead the plates into which you will add the cells (add 10 ml medium into each plate)
* Clear the cell medium- using a Paster pipet and the pump.
* Take 1 ml of PBS and use it to pill the cells from the plate's surface. If needed you can use an additional ml.
* Into the plates prepared ahead add ~20 ul drop. The remaining cell culture can be collected into a tube and be used for DNA extraction.
* Add 10 ml of prepared medium to each of the plates.
* For cells other the HEK-293, after clearing the medium add 5ml PBS, clear the PBS, and add 700ul-1ml of Trypsin, pill of the cells using the trypsin. Into each plate add 100-300ul of the cells in trypsin (the cell medium stops trypsin function)

**Change cell medium:**

Every 3 days or so it is needed to change the cells' medium:

* Clear the cell medium- using a Paster pipet and the pump.
* Add 10 ml of new medium

\*\* This will slow down the cells' growth rate, as when clearing old medium, growth factors that the cells have secreted are washed away.

**Use cells to extract DNA**

* Clear the cell medium- using a Paster pipet and the pump.
* Take 1 ml of PBS and use it to pill the cells from the plate's surface/as described for cells that needs trypsin.
* Into a 15 ml tube add the 2 ml of collected PBS + cells, and use them for the DNA extraction protocol- start by centrifuging 6 min in 250g. clear medium, resuspend cells.

**Freeze cells**

* Follow all steps of “use cells to extract DNA”.
* Up on centrifugation, clear medium and place them into serum+10% DMSO solution (premade, room temp). place in freezing tubes 1 ml of the solution (Cells + Freezing serum).
* Place in -20 1h, then liquid nitrogen for storage.
* **To unfreeze**: Defrost on ice for 1-3 minutes, then quickly centrifuge, remove serum + DMSO, resuspend in medium, place in premade plates.