Passaging of HeLa cells

For cell culture experiments, we cultured HeLa cells in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin (P/S). To maintain the culture, cells were passaged when confluent.

Material

Consumables

- Growth medium: DMEM supplemented with 10 % FBS and 1 % P/S
- Phosphate buffered saline (PBS)
- Trypsin/EDTA
- 100 mm cell culture dish
- 15 mL Falcon tube
- Serological pipettes (5 mL, 10 mL)
- Pipette tips (10 µL, 100 µL, 1000 µL)

Equipment

- Biosafety cabinet (use of laminar flow hood or clean bench is also possible)
- Centrifuge
- Pipettes

Procedure

All steps are performed in a sterile environment – we always worked within a biosafety cabinet, but using a laminar floow hood or clean bench is also possible.

1. Place the cell culture you want to passage under the sterile hood.
   Note: The cell culture dish is the only thing you do not clean with ethanol to prevent that ethanol is getting directly onto the cells.
2. Using a 10 mL serological pipette, remove the old cell culture media and discard it into a waste bottle.
3. Wash the cells with 3-4 mL PBS using a 5 mL serological pipette.
   Note: Do not add the PBS directly onto the cells but gently add it to the side of the dish.
4. Remove the PBS and discard it into a waste bottle.
5. Add 1.5 mL Trypsin/EDTA to the cells.
6. Incubate cells at 37°C for approximately 5 minutes until cells are detached.
7. Add 3 mL growth medium to stop the enzymatic reaction.
8. Pipette cells up and down a few times, thereby rinsing the surface of the cell culture dish.
9. Transfer cell suspension into a fresh 15 mL Falcon tube.
10. Spin down cells at 500 x g for 5 minutes at room temperature.
11. Discard the supernatant.
12. Resuspend cell pellet in 1 mL of growth medium by gently pipetting up and down.
13. Add 10 mL growth medium to a fresh 100 mm cell culture dish.
14. Add approx. 100 µL of cell suspension to new culture dish.
    Note: Using 100 µL means diluting the original cell culture 1:10. This will yield a confluent culture in about 3-4 days. Passaging volume can be adjusted according to your needs. If needed, cells can be counted so that a distinct cell number can be seeded.
15. Label cell culture dish: Cell type, passage number, date, name of operator (e.g.: HeLa, P5, 20.08.20, iGEM)
16. Incubate cells at 37°C, 5 % CO_2 and a humidified atmosphere.

Notes

- Always wear a labcoat and gloves.
- Clean the hood, your gloves and all the material which you are about to use with 70 % ethanol before starting your work/putting consumables under the sterile hood to prevent any possible contamination.
- Only open the consumables within a sterile environment to keep them sterile and to prevent any possible contaminations.