# Maintenance of cells

## Growth medium:

**CHO** (**C**hinese **H**amster **O**vary) cells:

Ham's F-12, with 2mM L-Glutamine, 10% FBS, 1% Pen/Strep, 100 µg/ml Zeocin™ ; PAA Laboratories®

**AAV** (**A**deno-**A**ssociated recombinant **V**irus) **293 cells**:

D-MEM (high glucose), with 2mM L-Glutamine, 10% FBS, 1% Pen/Strep, ; PAA Laboratories®

**HeLa** (Henrietta Lacks) cells:

D-MEM (high glucose), with 2mM L-Glutamine, 10% FBS, 1% Pen/Strep, ; PAA Laboratories®

**AAV-HT1080** cells:

D-MEM (high glucose), with 2mM L-Glutamine, 10% FBS, 1% Pen/Strep, ; PAA Laboratories®

## Selection medium:

CHO cells:

Ham's F-12, with 2mM L-Glutamine, 10% FBS, 1% Pen/Strep, Hygromycin B 550 µg/ml™ ; PAA Laboratories®

## Thawing cells:

All cells were stored in liquid nitrogen until ready to use.

- Remove cryovials from liquid nitrogen and thaw quickly in 37°C water bath.
- The outside of cryovial was decontaminated with 70% ethanol and the content of cryovial was transferred into a 15ml Falcon tube with pre-warmed growth medium.
- Centrifuge cells 100 x g for 5 minutes at room temperature and remove supernatant carefully.
- Resuspended cells were transferred in culture flasks ($75cm^2$) containing 15 ml pre-warmed growth medium and incubated in a 37°C incubator containing a humidified atmosphere of 5% $CO_2$.

## Medium change:

All media were changed after 48h.

## Passaging cells:

CHO cells:

- If cells reached a confluence of 80-90% the medium was removed and cells were washed twice with 5ml PBS to remove medium and serum.
- 2 ml trypsin were added to the cells and incubated for 5 minutes at 37°C. Checking for detached cells under the microscope and add 10ml of full medium to stop trypsination.
- Resuspend aggregated cells by gently pipetting.
- 1ml of suspended cells was transferred to new 75 $cm^2$ culture flask and 15ml fresh pre-warmed growth medium were added

AAV 293 cells:

- If cells reached a confluence of 80-90% the medium was removed and cells were washed twice with 5ml PBS to remove medium and serum.
- 2 ml trypsin were added to the cells and incubated for 1 minute at 37°C. Checking for detached cells under the microscope and centrifugation at 400xg for 3 min followed.
- 1ml of resuspended cells was transferred to new 75 $cm^2$ culture flask and and 15ml fresh pre-warmed growth medium were added

HeLa cells:

- If cells reached a confluence of 80-90% the medium was removed and cells washed twice with 5ml PBS to remove medium and serum.
- 2 ml trypsin were added to the cells and incubated for 5 minutes at 37°C. Checking detached cells under the microscope and adding 10ml of full medium to stop trypsination followed
- 1ml of resuspended cells was transferred to new 75 $cm^2$ culture flask and 15ml fresh pre-warmed growth medium were added

AAV-HT1080 cells:

- If cells reached a confluence of 80-90% the medium was removed and cells washed twice with 5ml PBS to remove medium and serum.
- 2 ml trypsin were added to the cells and incubated for 5 minutes at 37°C. Checking for detached cells under the microscope and addition of 10ml of full medium to stop trypsination followed

- Resuspend aggregated cells by gently pipetting.
- 1ml of suspended cells was transferred to new 75 $cm^2$ culture flask and 15ml fresh pre-warmed growth medium were added

## Freezing cells:

Cells were directly frozen in fresh growth medium (Ham's F-12, with L-Glutamin; PAA Laboratories) with 10% DMSO and a density of $1x10^7$ cells/ml.

- Determine total cell counts and calculate the volume of freezing medium to a yield of a final cell density of $1x10^7$ cells/ml.
- Centrifuge cells 100 x g for 5 minutes at room temperature and remove supernatant.
- Resuspend cells in the pre-calculated (90% growth medium+10%DMSO with final cell count of $1x10^7$ cells/ml) volume of chilled freezing medium. Aliquot cells into cryovials.
- Cells were frozen in automated controlled-rate freezing apparatus with a freezing rate of 1°C per minute to minus 80°C. For long term storage the cryovials were transferred into liquid nitrogen.