Subculturing for adherent cell lines

DMEM (-) = nothing added (can be substituted with 1x PBS)

DMEM (+) = added with 10% FBS, 1% L-Glutamine, 1% Penicillin/Streptomycin

* Protocol is for cells in a 90 mm dish/ T75 flask. Adjust accordingly for different sizes.

Remove and discard old media. Avoid disturbing the adhered cells.

Wash flask/dish with 10 ml of DMEM (-), then remove. Avoid washing off cells.

Trypsinize the cells by adding 2 ml of DMEM (-) and 1ml of trypsin. Swirl the flask/dish, then incubate for 4 – 7 minutes.

- Cells will die if exposed to trypsin for too long
- If cells don't get detached, use more trypsin (< 1 ml) and incubate for ~1 – 2 more minutes or tap the flask

Add 10 ml of DMEM (+) to stop the action of trypsin, then wash the flask/dish to wash off more cells. Transfer the cells into a 15 ml Falcon tube.

Spin down cells by centrifuging at room temperature (25^{o}C), 800 rpm for 5 minutes.

After centrifuging, pour out the media without disturbing the cell pellet.

Resuspend cell pellet with 1 ml DMEM (+) by pipetting up and down at least 10 – 15 times.

Split cells into new culture dish/flask at the desired and recommended density (usually ~30-40% for cell maintenance).