# Transferring cells using trypsinization

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1. Remove medium from a 100 mm plate (10 ml of cell culture). Save the medium for use in step 8.

2. Add 2 ml trypsin (0.05%) to rinse the plate. We use GIBCO Trypsin-EDTA (0.05%) with phenol red, or TrypLE Express, 1X; both are sold by Life Technologies/FisherThermo. We have so far found no functional difference between the two, save that TrypLE Express is stable at room temperature while Trypsin-EDTA is stored at 4° for up to about 2 weeks and at -20° for longer periods; TrypLE is more than twice as expensive as Trypsin-EDTA. The phenol red indicator will become pale when it comes in contact with the tissue-culture medium remaining in the plate; this is because the medium raises the pH (and inhibits the trypsin).

3. Immediately remove the trypsin.

4. Add 2 ml fresh trypsin. This time, the phenol red should maintain its color. Incubate at room temperature approximately 3 min. Note: The cells will tend to round up somewhat during this treatment.

5. Add 2 ml medium (or more) to inhibit the trypsin. Use the medium (including serum) in which the cells normally grow.

6. Using a Pasteur pipet, squirt medium from the plate at the cells to dislodge them from the substrate. If the cells are in a plate, it is usually possible to see them coming off the surface; the optics (and the geometry) are much less favorable in a T-flask. Check in an inverted microscope to confirm that the cells are in suspension.

7. Transfer the cell suspension to a centrifuge tube and spin to collect the cells.

8. While the cells are spinning, prepare a flask with the final volume of medium into which the cells are to be resuspended. This should consist of approximately 25% conditioned medium (the medium that was reserved in step 1 and 75% fresh, unused medium. Individual lines vary greatly in their need for conditioned medium, but it does no harm even when it is not required.

9. Remove the supernatant; add a small amount of medium from the flask (step 8), about 1 ml, pipet up and down with a Pasteur pipet to resuspend the cells, add the cell suspension back to the flask, and dispense into fresh plates.

10. Repeat the procedure when the cells reach confluence again.

Notes:

- Trypsinization is not required for most lines; we use it for only a few strongly adherent lines, mostly lines established in the Simcox lab. See notes on individual cell line webpages.

- This protocol is my modification of one from Amanda Simcox, and is given for a 100 mm plate (10 ml of culture). Use half of the listed volumes if you are working with a 25 $cm^2$ T-flask (5 ml of culture).