General Cell Culturing

- Culturing cells for general use (10 cm plate)
  1. Warm cell media and trypsin
  2. Aspirate cell media
  3. Wash cells with 5 mL of PBS 3 times
  4. Add 3 mL of trypsin to plate
  5. Place cells in incubator until cells are rounded and/or floating
  6. Use a P1000 set to 1000 µL to wash cells down off plate while it is tilted. Rotate plate to ensure all cells are washed off the plate
  7. Grab x volume of the trypsinized cells that will be kept for next generation.
  8. If the same plate is being reused:
     (a) Keep trypsinized cells in the pipet and set aside as not to contaminate the cells or allow the tip to touch anything
     (b) Aspirate out media from 10 cm plate
     (c) Add cells from pipet to 10 cm plate
     (d) Add 10 mL of appropriate cell media
  9. If a new plate is being used:
     (a) Transfer cells to new plate
     (b) Add 10 mL of appropriate cell media
     (c) Throw out the old cell plate
  10. Swirl plate in a infinity symbol pattern
  11. Update plate with new information (name, date, generation, cell type) if needed
- Splitting cells for live-cell imaging (35 mm plate)
  1. Aspirate media out of plate
  2. Wash cells with 5 mL of PBS 3 times
  3. Add 3 mL of trypsin
  4. Place cells in incubator until cells are rounded
  5. Use a P1000, set to 1000 µL , to wash cells down from tilted plate. Rotate plate to ensure all cells are washed off the plate
  6. Grab a 15 mL centrifuge tube
  7. Add 1 mL of trypsinized cells to this tube
  8. Add 1 mL of the appropriate cell media to this tube as well
  9. Mix by pipetting up and down
  10. Add 2 mL of the appropriate media to the 35 mm plate
  11. Add 40 µL of cells to the plate, making sure to disperse the cells throughout the plate when transferring from pipet
  12. Swirl plate in a infinity symbol pattern
  13. Update plate with new information (name, date, generation, cell type)