Seeding protocol (Tissue Culture)

Introduction

Plate the cells onto 24-well plate

Materials

- HEK resuspended in 10mL of media
- Complete media
- 24-well plate
- Hemacytometer cover glass
  - Brand name: Hausser Scientific
  - Found in the Weiss Common drawer
- p200 pipet tips

Procedure

Meassuring cell concentration

1. Add ~12-15uL of cells to the cell cover lid. WATCH OUT to no overflow the cells or too little cells. You want no bubbles either. If there are bubbles, use your finger and drag the glass oversheet to move the bubbles towards the edge or the place you pipetted stuff in.
2. Use the microscope to zoom in the 4x4 square
3. Count the number of cells in the 4x4 square using the counter (X)
4. Multiply X by 10^4 to get the concentration per mililiter (Y = XE4 cells/mL)

Plating

5. Each well needs: $5x10^4$ cells and the total volume is 500 uL
6. Calculate the seeding volume: Z = $5x10^4$ / (Y x 10^{-3} cells/uL) [2017 iGEM: Z = $2x10^4$ / (Y x 10^{-3} cells/uL]
7. Add (500 - Z) uL of media and Z uL of cells to each well
8. Gently shake the plate
9. Put in the 37°C incubator. The cells are ready for transfection at >75% confluency