# Seeding MCF-10A cells in Matrigel

1) Make bed of Matrigel in 8 wells of chamber slide
   You will need enough Matrigel for 8 wells
   Matrigel will be pre-thawed on ice (at least 20 min before use)

   45ul X 10 (8 wells plus 2 extra wells for pipetting error) = 450ul

   You will be given 1 ml aliquot
   Coat each well as described in demo by Grace and Eva
   Place in 37$^{o}$C $CO_2$ incubator for at least 30 minutes

2) Trypsinize cells
   You will be given a confluent 10 cm culture dish with MCF-10A cells
   These will be used to seed single cells in the 8-well chamber slide

   Aspirate medium
   Wash with 10 ml PBS
   Add 900ul of 0.05% Trypsin-EDTA
   Incubate at 37$^{o}$C for about 30 minutes

3) Make overlay medium while trypsinizing cells

   Will need 400ul/well
   Make 400ul X10 for 8 wells (+ 2 extra) – need 4 ml total

| Per well | X 10 wells |
|---|---|
| 400ul of Assay Media | 4 ml Assay media |
| 8ul of EHS (2% final) | 80ul EHS |
| 0.2ul of 10ug/ml EGF (5ng/ml final) | 2ul EGF(10ug/ml stock) |

   Original stock of EGF is 100 ug/ml – it will be given to you as a 1:10 dilution, i.e. 10ug/ml.

   4) Harvest trypsinized cells

   Resuspend in 5 ml of resuspension medium
   Spin cells at 900 rpm for 3 min

   Aspirate and resuspend in 1 ml of assay medium
   Pipette up and down with a 1ml tip to generate single cell mix (at least 5-10times)
   Add 7 ml assay medium and mix completely with a 10ml pipet

   Count cells (will be close to 1 million cells/ml)
   Calculate cells needed for 10 wells (8 wells plus 2 extra):
   6000 cells per well – 60,000 for 10 wells
   Add 60,000cells to 4ml of pre-made overlay medium in step3
   Mix completely

5) Add 400ul very carefully to each well with Matrigel bed

6) Incubate at 37$^{o}$C