**Invasion Assay (Transwell, Boyden Chamber)**

ADS, 4/17/17

Materials:

 24-well plate

 Transwell inserts (8µm pores)

 Matrigel (stored at -20°C, thawed on ice)

 Serum Free (SF) medium, type specific to cells

 Medium (+ optional chemo-attractant)

 4% formaldehyde in PBS

Protocol:

1. UV sterilize inserts for at least an hour.
2. Thaw Matrigel on ice. Make a 1:9 Matrigel:SF Medium solution on ice.
3. Pipette 100µL of Matrigel solution (for 24-well insert) into each transwell insert and let it set in the incubator or a 37°C oven. This will take 4-5 days in the incubator, but less time in a dry oven. The solution should be fully set and not flow when the plate is tilted.
4. In a new 24-well plate, pipette 500µL of media with optional chemo-attractant into the lower compartment of each well.
5. Using tweezers, carefully place 1 insert per well, and seed 100,000 cells/insert in the upper compartment in SF media.
6. Incubate for 24-72 hours. This will need to be optimized based on cell type, chemo-attractant and pore size.
7. At the end of the experiment, fix both top and bottom of insert with 4% formaldehyde and wipe the top of the insert with a cotton swab to remove cells and Matrigel from the top of the insert.
8. Count cells on the bottom of the dish and underside of the insert. Cells can also be immunofluorescently labeled or DAPI labeled at this point.

Depiction:



Keenan and Folch, ***Lab Chip***, 2008,**8**, 34-57