



## TITLE: INVASION ASSAY: Name: Rachana Ramachandran

### Experiment:

1. Culture HeLa cells in DMEM supplemented with 10% FCS and 1% PSA and glutamine at 37 °C in a CO<sub>2</sub> incubator.
2. Count and seed appropriate number of cells in 12-well plate (40,000 cells/well for 2 day and 80,000 cells for 1 day).
3. [In KD experiments (with ABR siRNA), 24-well plate was used (12,000 cells/well was added and after transfection with ABR siRNA, cells were kept for 72 hours at 37 °C in a CO<sub>2</sub> incubator.
4. Start overnight culture of bacteria in 3 ml of LB broth with appropriate antibiotics and incubate at 37 °C with no shaking.
5. Add bacteria from the overnight culture (at a ratio of 1:50) to warm plain DMEM and incubate for 3 hours at 37 °C in a CO<sub>2</sub> incubator.
6. Wash cells 2x with 1xPBS
7. Add 1ml of activated bacteria to each well and incubate for 90 min at 37 °C in a CO<sub>2</sub> incubator.
8. Remove the medium and add instead 1 ml DMEM supplemented with 100 µg/ml gentamicin (Cat#G1264, Sigma Aldrich) or without gentamicin (as control).
9. Incubate for 90 min at 37 °C in a CO<sub>2</sub> incubator.
10. Wash cells 3x with 1xPBS.
11. Lyse the cells with 1 ml lysis buffer containing 10 mM Tris (pH-7.4) and 1% TritonX100.
12. Mix the lysates well by few rounds of up and down pipetting.
13. Serially dilute the lysate to few different dilutions in 1xPBS (1:500; 1:1000; 1:2000; 1:4000; 1:8000).
14. From each dilution, 100µl were plated in LB-agar plates and incubated overnight at 37°C.
15. Count the number of colonies in each plate with colony counter.

### Quantification:

1. The concentration of protein in each sample is calculated by BCA assay kit method.
2. Number of colonies in each plate for cells treated with gentamycin (invaded bacteria) were counted and multiplied to its respective dilution to determine the cfu/ml (colony forming unit/ml). The number of colonies is divided with its protein concentration.
3. The number of colonies in all the plates, for cells not treated with gentamycin (total bacteria) serves as control (were equal in all cases,  $8 \times 10^6$  cfu/ml).