

## **Invasion assay protocol modified for HepG2 cells and *E. coli* expressing invasion protein**

(Yi Han, 16 Sept 2015)

### **Bacterial Infection Assay**

1. Prepare overnight cultures of BL21, and BL21+pSB1C3-inv plasmid (Inoculate 1 colony in 3mL LB, grow O/N in shaking incubator)
2. Seed 0.5mL of  $5 \times 10^5$ /cm<sup>3</sup> HepG2 cells per well in 24-well plates overnight, grow in CO<sub>2</sub> incubator. Check to see if cells are 90% confluent.
3. Grow bacteria under anaerobic conditions for 2, 4, and 6 hours, taking out aliquots at these timepoints and incubating on ice.
4. Remove DMEM media, wash cells with 500ul sterile 1XPBS. Do not pipette up and down when washing.
5. Add 400ul fresh warmed DMEM media+FBS without Pen-Strep. Work well by well to prevent drying out.
6. Take bacteria out of the shaking incubator, and mix by pipetting up and down. (Measure OD600 of 10X dilution.
7. Dilute bacteria with PBS to  $10^9$  cells/mL. Spin down 1mL and resuspend in PBS. Prepare 2X dilution and 4X dilution.
8. Inoculate 100ul of 2X dilution and 4X dilution of bacterial suspension to give an Moi of 200 or 100. Make sure pipette enters media, and move while releasing suspension. When done, swirl plate to ensure even distribution of bacteria.
9. Centrifuge the plate at 200-250g for 5 min. (centrifugation step not possible)
10. Infection for 3 hours in CO<sub>2</sub> incubator.
11. Remove supernatant, and wash once with PBS. Add 400uL fresh, warmed media with Kanamycin at 1000ug/mL.
12. After a further 1 hour of infection, remove media, wash cells 2X with 500ul PBS and lyse cells with 500ul PBS/0.2% Triton X-100.
13. Perform serial dilution of the cell cultures to  $10^3$  and plate out duplicates to determine number of intracellular bacteria.
14. Keep plates at room temperature, transfer to incubator to grow overnight. Count number of colonies and calculate CFU in the morning.