HOST-PATHOGEN INTERACTIONS LAB
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TITLE: ESPH TRANSLOCATION ASSAY:

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1) Seed HeLa cells in a 6-well plate.

When cells will reach ~70% confluency, infect the cells for 30 min (after 3h pre-activation) with the following bacterial strains: 1). Δ*espH*, 2). Δ*espH*+EspH-6xHis-SBP (-IPTG); 3). *escV*+EspH-6xHis-SBP (+IPTG); 4). Δ*espH*+EspH-6xHis-SBP (+IPTG). Induce EspH expression with 0.05 mM IPTG (For EspH, induction with 0.05mM IPTG yielded the highest effector protein expression).

IMPORTANT – Steps 2-9 should be done in the cold/ice.

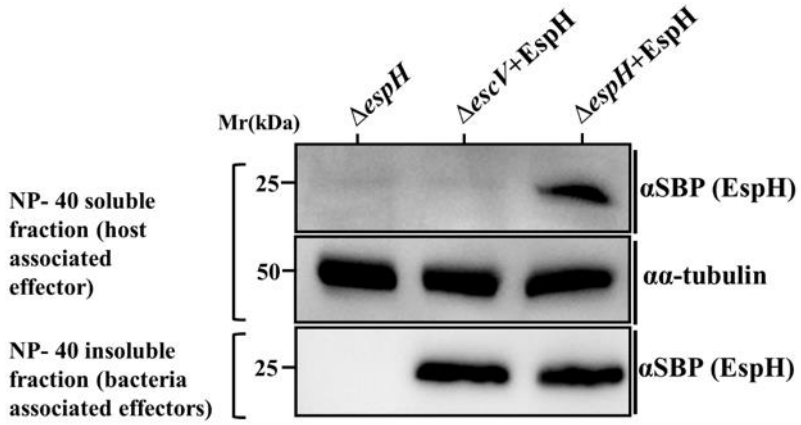
2) Wash infected cells 3x with ice-cold 1x PBS. After the last wash, aspirate the buffer as much as possible.
3) Add 0.2ml of ice-cold solubilization buffer to each well.
4) Scrape of the cells with 'Rubber Policeman' and transfer the lysate to a new ice-cold Eppendorf tube.
5) Leave on ice for 1 min
6) During that 1 min, mix the lysate by up and down pipetting 5-10 times gently with blue or yellow tip (w/o foaming).
7) Spin for 2 min at 4°C at max g (16,000g)
8) Collect the supernatant as much as possible while avoiding the pellet, and place it in an ice-cold Eppendorf tube. It is important to collect all the sup, because it contains the translocated effector, while the pellet contains the bacteria-associated effector.
9) Resuspend the pellet by multiple rigorous finger flicking until it is smeared on the wall of the tube.
10) Resuspend the pellet and supernatant in 100 µl of 4x SDS-PAGE sample buffer containing DTT/β-mercaptoethanol.
11) Heat 95 ºC for 5 min. For the pellet, sample buffer is added directly to the tube and boiled while for the supernatant, only the required amount to run in a gel is taken (10 or 15µl) at a time mixed with the sample buffer and boiled. The rest of the Supernatant is aliquoted and stored without adding sample buffer.

ESPH TRANSLOCATION ASSAY:

12) Run 13% gel; Western blot with anti-SBP to detect EspH in the two fractions. Probe the Supernatant (i.e. the NP-40 soluble fraction) with anti-tubulin for evaluating lysis levels.

Solubilization buffer

- 0.5% NP-40
- 10 mM Tris-HCl (pH-7.4)
- 100 mM NaCl
- 5 mM $MgCl_2$
- Protease inhibitor cocktail



Ramachandran et al IAI 2018