Injection into liposomes protocol

Protocol liposome injection experiment 1 - using GFP.

1. Start overnight cultures of strains in LB media containing 34µg / mL chloramphenicol. Incubate at 37°, 210 rpm.
2. Rehydration of lipids with PBS to a lipid concentration of 200 nM, vortex solution(s) for 30 seconds and incubate at room temperature for 30 min.
3. Measure optical density of overnight samples and dilute the overnight cultures to a optical density of 0.01.
4. Grow to an optical density of 0.4-0.6 (approx. 2 hours of incubation at 37°C at 210 rpm). This corresponds to the exponential growth phase of the bacteria. The strains are then induced with 1mM IPTG and 1% arabinose
5. Incubate of 320 µl bacterial solution and 80µl liposome solution together for 5 hours at 37°C at 210 rpm.
6. Separation of bacteria and liposomes: some samples by centrifugation and the others by filter centrifugation (filter size 0.45µm) for 2 min at 5000 rpm. Prior to any centrifugation half of the samples are incubated with 100 µM Proteinase K for 10 minutes
7. Measure the optical density (absorbance 600 nm) and fluorescence (excitation 395 nm & emission 509 nm) of 200 µl of the supernatants on a plate reader
8. Proceed with western blot of supernatants.

See the western blot protocol here.

Protocol liposome injection experiment 2 - using mCherry.

1. Start overnight cultures of strains in inducing LB media containing 34µg/ml Chloramphinicol, 1mM IPTG and 1% Arabinose. Incubate at 37° C, at 210 rpm.
2. Rehydration of lipids with PBS to a lipid concentration of 200 nM, vortex solution(s) for 30 seconds and incubate at room temperature for 30 min.
3. Incubate 250 µl of overnight bacterial solution and 50 µl liposome solution together for 3 hours at 37° C at 210 rpm.
4. Half of the samples are incubated with 100 µM proteinase k for 10 minutes.

5. Separation of bacteria and liposomes: half of the samples are centrifuged and the other half is filter centrifuged (filter size 0.45µm). Centrifuge for 2,5 min at 5000 rpm.
6. Measure the optical density (absorbance 600 nm) and fluorescence (excitation 587 nm & emission 610 nm) of 100µl of the supernates on a plate reader.
7. Proceed with western blot of supernates.

See the western blot protocol here.

Protocol liposome injection experiment 3 - using mCherry & GFP.

1. Start overnight cultures of strains in inducing LB-media with 34 µg / mL Chloramphinicol, 1mM IPTG and 1% Arabinose. Incubate at 37°, at 210 rpm.
2. Creating liposome solutions - Rehydrate premade lipids in PBS for half the samples and in aforementioned LB-media for the other half. Final lipid concentration of 300 nM. Vortex solutions for 30 seconds and incubate in room temperature for 30 min.
3. Measure optical density at 600 nm and fluorescence of GFP and mCherry of 100 µL of the cell cultures on a plate reader. mCherry excitation 587 nm & emission 610 nm. GFP excitation 395 nm & emission 509 nm.
4. Pellet cell cultures to remove LB-media supernate. Half of the samples are resuspended in 350 µL PBS. The other half in 350 µL of the same LB-media intended for overnight culturing.
5. Add 50 µL of the PBS liposome solution to the pellet resuspended in PBS, and 50 µL of the LB-media liposome solution to the pellet resuspended in LB-media. Incubation of the resuspended bacterial solution and 50 µl liposome solution together for 3 hours
6. All of the samples are incubated with 100 µM proteinase K for 15 minutes.
7. Separation of bacteria and liposomes. All samples are centrifuged at 5000 rpm for 2,5 minutes. Supernates is transferred to a filter eppendorf centrifugation tube (filter size 0.45 µm). Centrifuge for 1 minute at 5000g.
8. Measure the optical density at 600 nm and fluorescence of GFP and mCherry of 100 µL of the supernates on a plate reader. mCherry excitation 587 nm & emission 610 nm. GFP excitation 395 nm & emission 509 nm.
9. Proceed with western blot of supernates.

See the western blot protocol here.