The Spi$^-$ Assay of *gpt* delta Transgenic Mouse

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- Preparation of Plating Bacteria
    1. One day before the mutation assay, inoculate LB broth (5 ml x 2) with *E. coli* XL-1 Blue MRA or XL-1Blue MRA (P2) and incubate them overnight at 37°C with shaking.
    2. Next morning, inoculate LB + 0.2% (v/v) maltose with 1/100 vol. of the overnight cultures.
    3. Incubate at 37°C with shaking until OD_{600} reaches 1.0.
    4. Centrifuge and resuspend the pellets with 1/2 vol. of LB + 10 mM $MgSO_4$.
    5. Store the cells at 4°C until use (the concentrated suspensions of XL-1 Blue MRA and XL-1Blue MRA (P2) are used for titration and Spi$^-$ assay, respectively).

- *in vitro* Packaging Reactions

  <HOMEMADE Packaging Extract>
    1. Mix DNA samples by pipetting with wide-bore pipette (we use the product of QALITY SCIENTIFIC PLASTICS, QSP #: 118-96RN).
    2. Thaw SE at room temperature and then thaw FTL with finger tips. Thaw each one of SE and FTL for every two packaging reactions.
    3. Immediately aliquot half (15 µl) of FTL into a fresh 1.5-ml eppendorf tube.
    4. Add the DNA sample (5 ~ 7.5 µl) to the 15 µl of FTL.
    5. Mix by pipetting with the wide-bore pipettes about 20 times, avoiding introduction of air bubbles.
    6. Add SE (30 µ l) with the wide-bore pipettes. Mix well.
    7. Incubate the tubes at 37°C for 90 min. The remaining SE (30 µ l) and FTL (15 µ l) are frozen again on dry ice.
    8. Thaw re-frozen FTL (15 µ l), and add it to the reaction and mix several times.
    9. Thaw re-frozen SE (30 µ l), and add it and mix several times.
    10. Incubate at 37°C for an additional 90 min.
    11. Add SM buffer and adjust the final volume to 300 µl. Mix by gentle vortexing.
    12. Keep them at 4°C or use immediately.

< Transpack Packaging Extract (STRATAGENE, La Jolla, CA) >

(See the original protocol.) Adjust the final volume to 300 μl by SM buffer.

- Titration of the Packaged Phage with Strain XL-1 Blue MRA

1. Take the packaged phage suspension (5 μl) and mix with LB broth (495 μl) to make a 100-fold diluted suspension.
2. Add 10 μl of the diluted suspension to 200 μl of the 2-fold concentrated XL-1Blue MRA culture. We use sterile glass tubes (ϕ 1.2 cm x 7.5 cm) for this assay.
3. Incubate for 20 min at room temperature.
4. Add 2 ml of molten λ-trypticase soft agar.
5. Pour on λ-trypticase agar plates and incubate overnight at 37°C.
6. Count the number of plaques and calculate the number of p.f.u. (plaque forming unit) per packaging reaction.

[Titer = Number of plaques (average of 2 plates) × Dilution Factor]

Dilution Factor = 3000

- The Spi assay of the packaged phage using XL-1Blue MRA(P2)

1. Add 150 μl of the remaining packaged phage suspension to 0.5 ml of the 2-fold concentrated XL-1Blue MRA(P2) suspension. We use 2 sterile glass tubes (ϕ 1.2 cm x 7.5 cm) for this assay.
2. Mix by gentle vortexing.
3. Incubate for 20 min at room temperature.
4. Add 2 ml of molten λ-trypticase soft agar to each tube.
5. Pour on λ-trypticase agar plates (ϕ 9 cm x 2 plates).
6. Immediately, expose the top agar layer to gas fire for 1-2 seconds. This is for removing the bubbles on the agar layer.
7. Incubate overnight at 37°C.
8. Count the number of clear plaques on the 2 plates (Spi^- candidates).

- Confirmation of the Spi$^-$ phenotype

1. Punch out the clear plaques (Spi$^-$ candidates) with sterilized glass capillary (Clay Adams, disposable Micropet 100 μl).
2. Suspend the agar plug in 50 μl of SM buffer.
3. Spot 20 μl of the suspension each on two λ-trypticase plates where either XL-1 Blue MRA (P2) or XL-1Blue MRA is spread with λ-trypticase soft agar.
4. Incubate overnight at 37°C.
5. Count the number of real Spi$^-$ plaques. If the Spi$^-$ candidates are real, they should make clear spots on both MRA (P2) and MRA plates.
6. Calculate the mutant frequency by dividing the total number of real Spi$^-$ plaques by the number of total plaques recovered.

- Preparation of the Lysate of the Spi$^-$ Phage

1. Inoculate LB broth with *E. coli* LE392 and incubate overnight at 37°C with shaking.
2. Spin down the overnight culture and suspend the pellet in 1/2 vol. of LB + 10 mM $MgSO_4$.
3. Punch out the clear spot (the real Spi$^-$) on agar plates with capillary and add the agar plug to 50 μl of the *E. coli* suspension.
4. Stand for 5 min at room temperature.
5. Add 2 ml LB broth + 10 mM $MgSO_4$.
6. Incubate at 37°C with vigorous shaking until lysis occurs (about 7~8 hours).
7. Add 100 μl chloroform.
8. Centrifuge at 5, 000 rpm for 10 min.
9. Take the supernatant and keep it at 4°C.

- Check the Titer of the Spi- Lysate

1. The lysate (10 μl) is diluted 10^6 fold with LB broth.
2. The diluted lysate (10 μl) is mixed with XL-1 Blue MRA (200 μl of the 2-fold concentrated overnight culture).
3. Add 2 ml of molten λ-trypticase soft agar.
4. Pour on λ-trypticase plates.
5. Incubate overnight at 37°C.
6. Count the number of plaques.
7. Calculate the p.f.u. per ml of the lysate.

- λ-trypticase agar plate (1 L)

BBL trypticase peptone (Becton Dickinson) 10 g

NaCl	5 g
Agar (Difco)	10 g
H_2O	1 L

Agar conc. is 1%. The plates should not be dried. Fresh and wet plates are preferable. The volumes of agar plate are about 25 ml (Eiken Sterile Auto Schale, ϕ 9 cm).

- λ-trypticase top agar (100 ml)

BBL trypticase peptone (Becton Dickinson) 1 g

NaCl	0.5 g
Agar (Difco)	0.6 g
H_2O	100 ml

It will be very difficult to identify Spi- plaques after overnight incubation if there are small bubbles on the top agar layer. Thus, small bubbles on the top agar layer should be completely removed out as soon as pouring the molten soft agar on the plates. It can be done by exposing the top agar layer to gas fire 2 - 3 seconds. Exposure of the agar plates containing phage and bacteria to gas fire for 2 - 3 seconds does not affect the Spi- formation.