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Propagating Serratia phage 92A1 and host

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We use this protocol and it's working

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Abstract

This is a protocol for propagating *Serratia* phage 92A1 and its host, *Serratia* strain 92. *Serratia* phage 92A1 (Genbank OR088902.1) is a 174 kb, T4-like phage with a probable arabinose-based modification of its genome. You can use this protocol to generate large quantities of phage for DNA extraction and nucleoside chemical analysis.

Propagating *Serratia* strain 92

1 Streak culture out on an LB agar plate and incubate at room temperature (25 °C) for two days. You should see the formation of light tan colonies that have a slightly gooey appearance.

Note

Short-term storage: You can store this plate at 4 °C for several weeks.

2 Set up a liquid culture of the host by inoculating 1 mL of LB broth with a single colony and incubating in a shaker at room temperature (25 °C) shaking at 200 rpm. Grow for two days.

Note

Long-term storage: You can freeze down and store this liquid culture at −80 °C in 20% glycerol.

Propagating phage 92A1

3 Infect 200 µl of host liquid culture with 10 µl of phage in a microcentrifuge tube. Let incubate on benchtop for 10 minutes.

Note

For maximum phage recovery, use a concentration of phage that will give you confluent lysis of the full plate. We determine this by titering the phage beforehand. To get more phage out of your propagation, you can repeat this process to make multiple phage plates. We often harvest DNA from three plates of phage.

4 Move 3 mL of molten 0.25% top agar (see recipe below) into a glass culture tube. Once the top agar is warm but not so hot that it will kill your cells (aim for lower than ~45 °C), add the pre-mixed phage and host to the tube.

Note

0.25% top agar
- 100 mL LB
- 0.25 g agarose
- 100 µl 1M $MgSO_4$

Microwave or autoclave to dissolve agarose. Keep molten in a heat bath before use.

5 Rapidly pour the inoculated top agar onto a room-temperature LB plate, and swirl to distribute a thin layer of top agar across the surface of the plate. Put it down and let it fully solidify. Do not disturb the plate until it is fully solidified.

6 Incubate at room temperature (25 °C) for one day. You should see small dark plaques form, and if the input concentration of phage is correct, these plaques should merge to form a confluent zone of lysis.

7 To harvest the phage, flood the plate with 3 mL of SM buffer (recipe below) and incubate on benchtop for 10 minutes. This allows the phage to diffuse out of the top agar into the buffer layer, which can then be harvested.

Note

SM buffer
- 50 mM Tris-HCL, pH 7.5
- 100 mM NaCl
- 8 mM $MgSO_4$
- 0.01% gelatin

Alternatively, you can purchase from Bioworld (41920012).

8 Pipette the SM buffer with phage into a 15 mL conical tube, and add 300 µl of chloroform to the mixture. If you are combining multiple plates worth of phage, scale up the amount of chloroform you are using (300 µl for every 3 mL buffer).

9 Mix buffer and chloroform on a rocking shaker for 10 minutes. Use Parafilm to seal the top to make sure nothing leaks out.

10 Spin down at 15,000 × g for 5 min. You should see the cells and other debris collect in a cohesive opaque layer above the chloroform.

11 Collect the supernatant. If it's still cloudy, repeat the chloroform treatment and spin. Keep doing this until the supernatant is clear.

12 Store the supernatant on 50 µl of chloroform in the fridge at 4 °C. This phage appears to be stable long term at 4 °C, but consider checking it every 6–9 months to make sure it's viable, and propagate it if you are losing titer.