SEA PHAGES

New Host Data Collection

General Information

Investigator Information

Last name _____

First name _____

Institution _____

Best contact email address _____

Which host bacterium did you test?

Did you find any phages that infected this host bacterium?

If you answered yes to the previous question, are these phages entered on Phages DB?

- ❍ entered in Phages DB with complete information
- ❍ entered in Phages DB but with incomplete information
- ❍ not entered in Phages DB

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Growth and Storage Conditions

Please name media and list additives, in all combinations, used for **liquid** media.
[e.g. LB media, supplemented with 1 mM $CaCl_2$, 10 µg/ml carbenicillin, 0.05% Tween]

Please name media and list additives, in all combinations, used for **top agar** in plates.
[e.g. LB media, supplemented with 0.4% agar, 1 mM $CaCl_2$, 10 µg/ml carbenicillin, 0.05% Tween]

Please name media and list additives, in all combinations, used for **base agar** in plates.
[e.g. LB media, supplemented with 1.5% agar, 1 mM $CaCl_2$, 10 µg/ml carbenicillin, 0.05% Tween]

Please indicate incubation temperatures and growth times for colony formation, saturated liquid culture formation, and bacterial lawn formation.
[e.g. colony formation: 37 °C, 2 days]

colony formation _____
saturated liquid culture formation _____
bacterial lawn formation _____

Did you test to correlate OD600 with colony forming units (cfu)?

❍ yes
❍ no

If you answered yes to the previous question, please provide a description of the correlation between OD600 and cfu.
[e.g. OD600 of 0.1 = 4e7 cfu/mL]

If you have determined the doubling time of the host, please provide information here. If you have completed a growth curve, please attach graph(s) using the instructions provided at the end of the form.

Please provide a description of colonies and liquid cultures. [e.g. colonies were mucoid, liquid cultures were clumpy]
Please upload images using the instructions provided at the end of the form.

How often did you start a liquid culture for plating use?
Please comment on the robustness of lawn formation of a given culture over time.

How did you store your working culture?
[e.g. streak on plate at 4 ºC]

Please describe any issues you may have encountered with contamination.

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Phage Isolation

What was your source of phage?
[e.g. soil next to compost]

What was your success rate for plaque formation from **direct plating**?
[e.g. 25%, that is 4 out of 20 attempts of direct plating resulted in plaque formation]

Did you use enrichment?
- ❍ I successfully used enrichment
- ❍ I was not successful with enrichment
- ❍ I did not use enrichment

If you used enrichment, what was your success rate for plaque formation **after enrichment**?
[e.g. 50%, that is 5 out of 10 attempts of plating after enrichment resulted in plaque formation]

If you used enrichment and your enrichment protocol is different from that provided in the Resource Guide, please provide details of your protocol.

How long did it take for plaques to form?

Please describe plaque morphologies. [e.g. large and clear]
Please attach images of plaques using the instructions provided at the end of the form.

If you incubated using different medias and/or temperatures, please provide information below on differences in plaque morphology and/or time to plaque formation.
[e.g. incubating plates at 37 ºC resulted in 3-fold more plaques that were also larger than when incubating plates at 30 ºC]

If you used an alternative phage buffer to that described in the Resource Guide, please provide details of the buffer.

What were the titers of your prepared lysates? Provide answers as pfu/mL.
Please describe any modifications to the protocol that allowed you to obtain a high phage titer.

Did you notice any correlation of variability in titer (pfu) with **age of phage lysate (phage in buffer)**? If so, please provide information below.
[e.g. a 2-fold decrease in pfu was observed when using a 1-month old phage lysate compared to a 1-week old phage lysate]

Did you notice any correlation of variability in titer (pfu) with **age of culture**? If so, please provide information below.
[e.g. a 2-fold decrease in pfu was observed when using a 2-week old culture compared to a 2-day old culture]

Please list any general observations and/or recommendations for phage viability and storage conditions.

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DNA Extraction and Analyses

Please comment on any issues you might have encountered in amplifying phage for genome extraction.
Please include any modifications you might have employed, including those that did not work well.

Were you able to successfully extract the genome from phage isolated using the new host?

- ❍ yes
- ❍ no

If you answered "yes" to the previous question, was the genome sequenced?

- ❍ yes
- ❍ no

Please describe any issues you may have encountered during genome extraction.
If you used an alternative genome extraction protocol to that described in the Resource Guide, please provide details of the protocol below.
Please include any modifications you might have employed and the reason(s) for such modifications.

Did you perform DNA restriction analyses?
If yes, please indicate restriction enzymes used and attach images of gels using instructions provided at the end of the form.

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Summary, Citations and Attachments

Please provide information on practices that worked well for you that were not already requested in this form.

Please describe challenges you faced working with the host and any recommendations you might have for those piloting new hosts.

Please cite any primary literature or other resources referenced in working with the new host.

Please use the space below to share your thoughts on how the survey could be improved.

Finally, but most importantly, we'd like to thank you for your time and effort. The information you have provided us will be reviewed to identify best practices for phage-hunting across the SEA. The information gathered here will become available for all phage-hunters via phagesDB.

Thank you.

The SEA-PHAGES Team.

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(End of Part 5/5)