Molten Streaking for Singles: rapid tube-free serial purification of viruses

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ABSTRACT

This protocol presents a simplified approach for purification of viral strains directly from plaques without the need for preparation of dilutions. Using the 'Tube-free agar overlay' approach viruses are 'picked' directly from source plaques with a toothpick and streaked into molten host lawns in a manner directly analogous to re-streaking based colony purification of bacteria. This approach requires less time and material than dilution based approaches and yields equivalent purification of viruses. Formation of plaques along streaks also offers further confidence that plaques selected for further purification derive from an isolated phage particle rather than a splash containing multiple viruses or a prophage induction event.

A video demonstrating the approach is available as supplementary material with the original publication (direct link here).

EXTERNAL LINK

https://doi.org/10.1016/j.mex.2018.01.007

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Kauffman, K. M. & Polz, M. F. Streamlining standard bacteriophage methods for higher throughput. MethodsX 5, 159–172 (2018). https://doi.org/10.1016/j.mex.2018.01.007 - This protocols.io protocol has been adapted from the MethodsX article, which is published under the Creative Commons Attribution License (CC BY).

Kauffman and Polz - 2018 - Streamlining standard bacteriophage methods for hi.pdf

PROTOCOL STATUS

Working

GUIDELINES

Please also refer to the original publication for a **"Microreview of additional considerations relevant to agar overlays"**, which highlights methodological aspects that are known or expected to impact the nature, number, and diversity of viruses recovered using agar overlay approaches.

MATERIALS TEXT

See "Guidelines & Warnings" section for materials to have ready before start.

BEFORE STARTING

Materials to prepare in advance for agar overlay approaches

Host culture: This procedure works well with 100uL of overnight host culture for each standard size (90mm) petri dish.

Virus material:

- For ***Serial Purification of Viruses*** the starting material may be an agar overlay plate containing a plaque to be purified, or may be a liquid or frozen stock of unknown titer - such as, for example, an archived plaque or plaque-eluates generated using the 'Archiving Plaques' protocol.

Bottom agar plates: Prepare 1.0% agar containing media ('bottom agar') in a glass bottle or flask with a stirbar and sterilize, pour 25mL per standard size petri dishes, and allow to solidify.

Top agar bottle: Prepare 0.3% agar containing media ('0.3% top agar') in a glass bottle with a stirbar and sterilize; though each plate will require only 2mL of top agar, volumes of up to 500mL can be prepared and re-used across multiple days of plating.

I. Setting up the top agar in a beaker-waterbath

1 **Prepare a beaker-waterbath:** Place bottle of top agar into a glass beaker and add water to the beaker up to the level of the top agar in the bottle; for example, a 500mL glass bottle into a 1L beaker.

2 **Melt the top agar:** Place the beaker-waterbath with the top agar bottle into a microwave and cook without boil-over until thoroughly melted.

> NOTE
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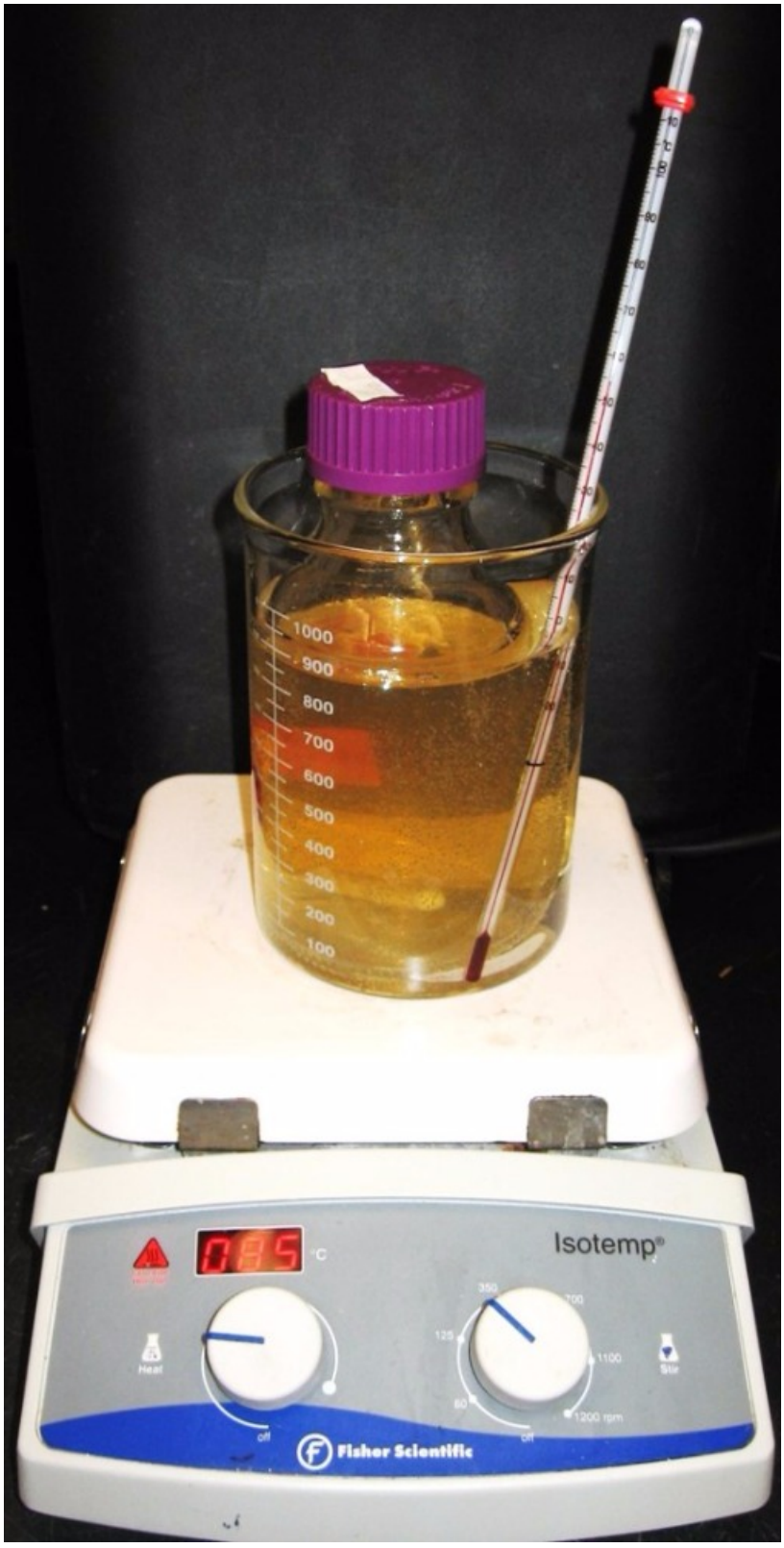
> *Note:* It is exceedingly important to achieve a 'smooth melt' of the top agar to ensure that plaques will form and be visible. Media composition affects the time it takes to achieve a ‘smooth melt’, however a general approach is as follows: start by melting the top agar for several 5-minute cycles at low % power, once the top agar appears nearly completely melted increase the % power and cook until the top agar comes to a boil, observe the top agar during high power cooking to prevent boil-over, bring to a boil 3 times.

3 **Equilibrate the top agar to 50-52°C:** Place the beaker-waterbath with the top agar onto a hot plate with stirring, place a thermometer into the beaker-waterbath and leave it there to wait for equilibration to 50-53°C (Figure 1).



NOTE

Note: This will require setting the heat block to a temperature greater than the target temperature, for example up to 85°C, but this is dependent on specific heat block models and must be determined by the user.

II. Streaking for singles: rapid serial purification of viruses from plaques - **Streak 1 (Day 1)**

4 **Prepare the molten agar overlay:** Have all materials ready to advance to Streak 1 steps (Steps 5, 6, 7, & 8) before starting this step as

the lawn cannot be allowed to solidify before the streak.

5 **Add host -** Pipette 100uL of overnight host culture directly onto the bottom agar

6 **Add molten top agar -** Pipette 2mL of molten top agar from the bottle directly to the bottom agar next to the hosts using either a 5mL pipette or a serological pipette

7 **Make overlay -** Swirl vigorously but briefly to mix the bacteria and molten top agar and spread it across the plate.

8 **Streak viruses -** from the source plate directly into the still-molten top agar (link to demonstration Video 1 is here) such that when the host lawn forms it will contain single colonies; it is necessary to do this swiftly such that streaking is completed before the agar solidifies.

- Insert a sterile toothpick (or pipette tip) into the source plaque or solution
- Swirl the toothpick into a small area of still-molten agar overlay
- Use a second toothpick to make three separate strokes in the same direction through the still-molten top agar, each time passing through the area where the first toothpick was touched.
- Use a third toothpick to make a repeating Z-stroke through the still-molten top agar, first passing once through the streaks from the second toothpick.

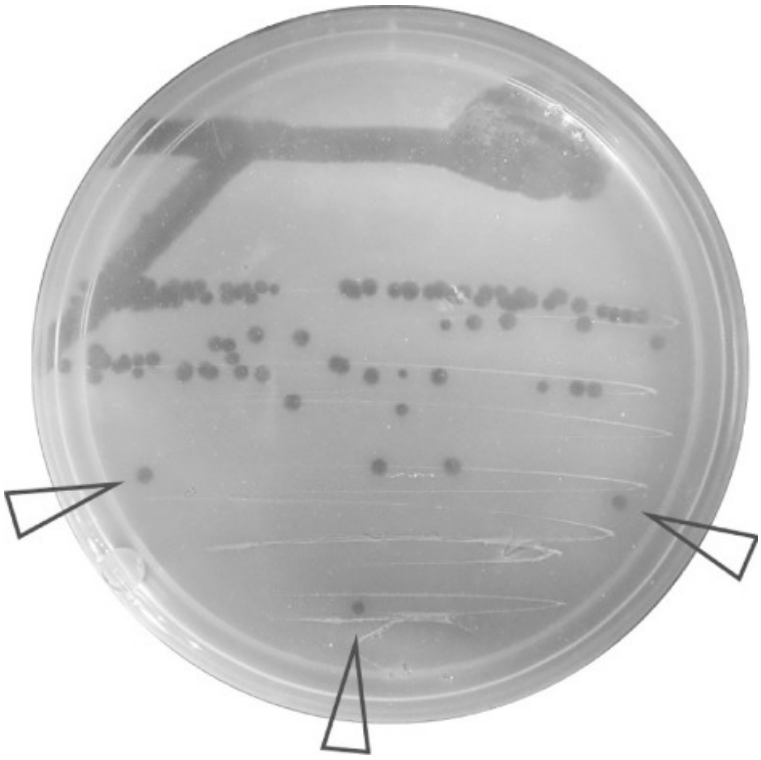
NOTE

Note: If the starting material is a liquid stock then drop up to 20 ul of the sample into one spot in the molten overlay and streak as described. If the starting material is a frozen stock then collect a small mass of that stock with a sterile toothpick or tip and transfer into one spot in the molten overlay and streak as described. Take care to ensure that frozen stocks remain frozen during the procedure to prevent loss of viruses sensitive to freeze-thaw cycles.

9 **Allow agar overlay to solidify:** Leave the plate on the bench, top agar side up, for 20 minutes to allow the agar to completely solidify, then place in desired incubation conditions and monitor for plaque formation.

NOTE

Note: Some plaques may take days or weeks to form (see original publication for discussion and reference), if keeping plates for extended periods of time to monitor for plaque formation ensure that the plates are protected from dessication.



III. Streaking for singles: rapid serial purification of viruses from plaques - **Streaks 2 & 3 (Days 2 & 3, or later)**

10 Once plaques appear on the first plate, use this plate as a source, insert a sterile toothpick into a single plaque and repeat the streaking for singles approach for the desired number of purifications. Single plaques arising from the final streak can be picked and archived, or used to generate large-scale liquid or plate lysates of the purified virus.

NOTE

Note: When isolating from a plaque, ensure that the plaque is well separated from other plaques (such as those as indicated by the arrows in Step 9).

NOTE

Note: It is common practice to consider plaques generated from the third streak as “purified”. When deciding on number of serial purifications consider that use of fewer serial passages may result in recovery of mixed stocks of viruses but may also minimize changes in viruses resulting from adaptation or selection on the host during passage.