Spot-on-lawn (halo) assay for screening enrichment cultures and isolates for viruses

Kenneth M. Stedman, Kate Porter, and Mike L. Dyall-Smith

Abstract

This protocol is based on Schleper et al. (1992) as modified by Stedman et al. (2003).

This is a protocol from:

Stedman, K. M., K. Porter, and M. L. Dyall-Smith. 2010. Chapter 6: The isolation of viruses infecting Archaea. Manual of Aquatic Viral Ecology. Waco, TX:American Society of Limnology and Oceanography. doi:10.4319/mave.2010.978-0-9845591-0-7

Please see the published manuscript for additional information.

Citation: Kenneth M. Stedman, Kate Porter, and Mike L. Dyall-Smith Spot-on-lawn (halo) assay for screening enrichment cultures and isolates for viruses. **protocols.io**
dx.doi.org/10.17504/protocols.io.eagbabw
Published: 09 Dec 2015

Guidelines

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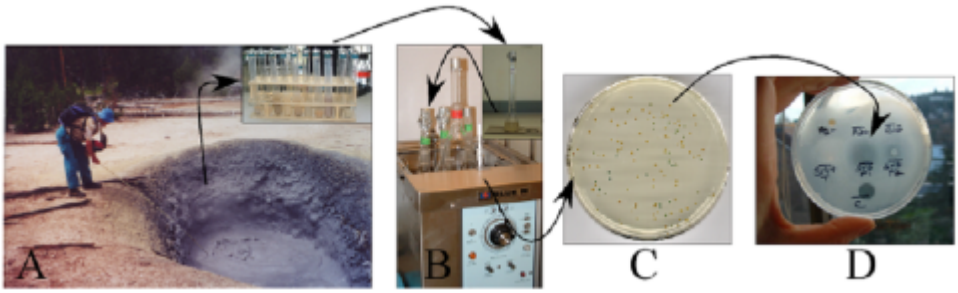

Fig. 1: Pictorial overview of isolation of *Sulfolobus* viruses.
(A) Wolfram Zillig sampling at a typical *Sulfolobus*-containing pool in Yellowstone National Park, USA, September 2000 (inset shows anaerobic tubes with samples).
(B) 80°C incubator with long-necked growth flasks (detail in inset).
(C) Singlecolony isolates of *Sulfolobus solfataricus* on a Gelrite® plate. This plate contains a mixture of *S. solfataricus* containing (blue colonies) and lacking (brown) a vector expressing the *lacS* gene from *S. solfataricus* and was sprayed with 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal) (see Jonuscheit et al. 2003).

(D) Lawn of *S.solfataricus* strain P1 with halos of growth inhibition due to virus production by 2-µl spots of virus-infected strains. Spots labeled SV2P1 and SV2P2 are from *S. solfataricus* strains P1 and P2 infected with SSV-I2 respectively (Stedman et al. 2003). Spot labeled C is a detergent-positive control. Spot labeled P2- is uninfected *S. solfataricus* strain P2 as a negative control.

Protocol

Step 1.
Gelrite plates are preincubated ca. 10 min at 80˚C to dry.

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Step 2.
10 mL of Sulfolobus medium with ca. 0.2 % (w/v) Gelrite is boiled to dissolve the Gelrite.

Step 3.
This “softlayer” is allowed to cool slightly (to ca. 80˚C).

Step 4.
Approximately 3 mL of softlayer are added to ca. 0.2 mL of exponentially growing host cells, generally *Sulfolobus solfataricus*, and spread on a plate by swirling.

Step 5.
After the Gelrite solidifies, 1–2 µL of culture or supernatant to be screened is spotted on the plate.

Step 6.
For a positive control, 1 µL of a 0.01% (v/v) Triton X-100 solution is spotted.

Step 7.
Plates are incubated as above for 2–3 d and plates examined for clearing around spots (Fig. 1D in guidelines).

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12:00:00