Isolation of Haloviruses from Natural Waters

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Abstract

This is a protocol from:

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Please see the published manuscript for additional information.

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Guidelines

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Modified growth medium (MGM) contains 5 g peptone and 1 g yeast extract per liter of salt water. The salt concentration is varied according to the host strain, and is detailed in the Halohandbook.

Protocol

Step 1.
Salt lake samples are collected from hypersaline waters, which typically range from 15% w/v total salt, up to saturation (ca 35%).

Step 2.
Samples are collected in sterile 5–10 mL vessels and may be stored for several weeks at room temperature.

Step 3.
In the laboratory, cells and cellular debris are removed by centrifugation (5,000g, 10 min, room temperature).

DURATION
00:10:00

Step 4.

The supernatant is then screened directly for viruses by plaque assay.

NOTES

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The use of chloroform is avoided, because it is known to have a detrimental affect on both phage-like and lipid-containing haloviruses.

Step 5.

The choice of host strains depends on the experimental objectives, and includes well-characterized members of the Halobacteriaceae, such as *Hbt. salinarum* (host for ΦH and several others) or *Har. hispanica* (host for SH1, His1, His2, and others), or natural isolates from the same source, such as *Hrr. coriense* (host for HF2).

NOTES

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To maximize isolation success, several hosts should be used for the same sample. The advantage of the use of characterized hosts is that methods for genetic manipulation are often established and their genome sequences have been determined.

Step 6.

Base and overlay plates (90 mm) are made with MGM (see guidelines), solidified using 15% w/v agar.

Step 7.

A range of salt water concentrations should be examined, because salt concentration seems to greatly affect the size and clarity of plaques.

NOTES

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Using salt water concentrations that are 2% to 5% lower than the optimum for host growth commonly gives better plaques.

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Incubation temperature is also important, because some haloviruses plaque poorly or not at all at 37–42°C, whereas they form clear plaques at 30°C.

Step 8.

Plates can be stored indefinitely at 4°C (wrapped in plastic to prevent dessication), but should be warmed to room temperature or warmer for use.

Step 9.

For virus isolation, 100–500 µl of the cleared water sample is mixed with 150 µl of exponentially growing host cells.

NOTES

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These may be characterized strains of haloarchaea, or natural isolates, for example, isolates from the natural water sample.

Step 10.

Then, 3–4 mL of molten (50°C) top-layer MGM (with 0.7% w/v agar) is added, and the solution mixed gently and poured evenly over the plate.

Step 11.

After setting on a level surface for 5–10 min, plates are incubated aerobically, inverted in airtight containers at 30°C and 37°C for 1–4 d, and checked every day for plaques.

DURATION

00:10:00

Step 12.
Any visible plaques are picked using sterile glass Pasteur pipettes, or sterile plastic micropipette tips.

Step 13.
These agar plugs are then transferred to tubes containing 500 µl of halovirus diluent:

2.47 M NaCl
90 mM $MgCl_2$
90 mM $MgSO_4$
60 mM KCl
3 mM $CaCl_2$
10 mM Tris-HCl pH 7.5

Step 14.
They are then vortexed to homogenize the sample.

Step 15.
These suspensions are then replaqued on overlay plates to purify the isolates and to eliminate “false plaques” caused by artifacts in the agar overlay or contaminants in the water sample.