Coring Technique

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Abstract

This is a technique for isolating phage from agar plates. When working with lytic phage, well-isolated, cleared areas in a lawn of bacteria (called plaques) are removed from the agar (by coring with a sterile pipette) and used to infect fresh bacteria in liquid medium. The lysed culture can be used for a new plaque assay after 0.2 um filtering to remove residual bacteria. New phage are "plaque-purified" a minimum of three times before they are considered to be free of other phage.

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Protocol

Step 1.
Poke the plaque in the agarose with the tip of an autoclaved Pasteur pipette to core it out drawing agarose chunks into the pipette

NOTES
Bonnie Poulos 17 Jun 2015
I would pick a few (2-4?) plaques just in case one does not lyse

ANNOTATIONS
Chris Upton 10 Sep 2015
Can you use sterilized tooth-picks?
Would be cheaper.

Step 2.
Inoculate 2-5 ml dense cells (whatever is available) with the cyanophage plaque by pipetting up and down a few times

Step 3.
Let this sit in the incubator for 60 minutes to adsorb and then dilute with about 25 ml media

DURATION
01:00:00

Step 4.
Do this in parallel to a control tube of just cells and observe the cyanophage inoculated cultures for lysis (clearing) relative to the controls