

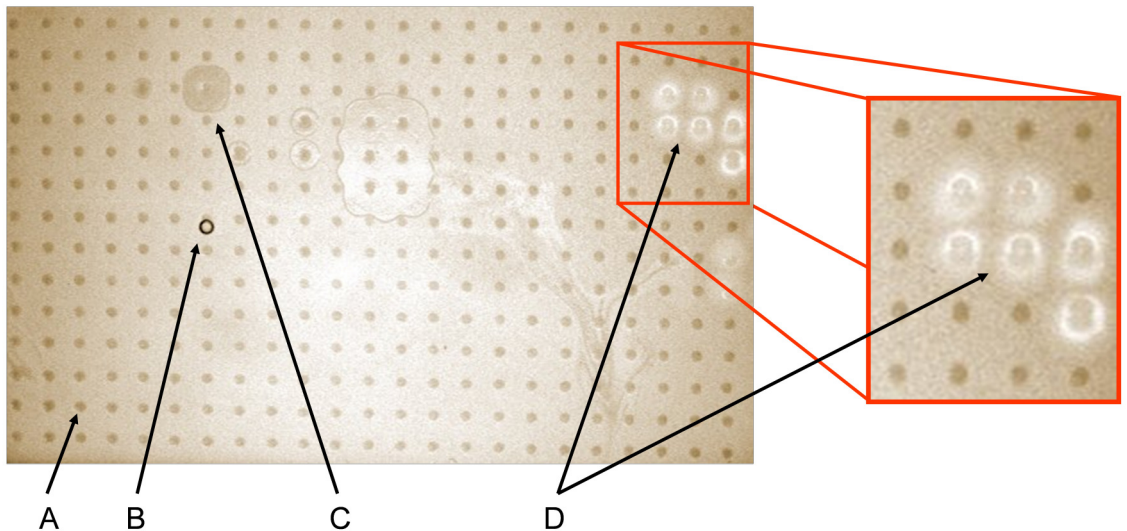
### Assay for T1-Phage contamination

T1-bacteriophage contamination is present in many BAC clone resources. This agent can cause the E.coli host cells to lyse and no longer propagate in culture. Phage T1 contamination can spread rapidly to other E.coli cultures as well. Procedures must be in place to minimize the chance of transfer of lytic phage contamination to other clone collections by applying appropriate microbiological practices to ensure that your labs remain contamination free. **PLEASE NOTE** that even clones which have tested negative for phage should still be handled with care, as no phage assay can be guaranteed to be 100% accurate.

- 1 Prepare thin LB+agarose plates as per above protocol, but without antibiotic. Also prepare 500ml bottle of top-agar (LB + 0.8% agarose in a bottle). Store plates at 4°C, inverted, for up to 2 days if not used immediately.
- 2 The evening before phage testing, prepare a 200ml LB (-) antibiotic culture of DH10B cells (Invitrogen). Grow O/N at 37°C in shaking incubator.
- 3 Remove the culture of DH10B from the incubator and keep at room temperature until ready to use.
- 4 Melt a 500ml bottle(s) of LB top-agar in microwave and place in a 42°C water bath. Allow cooling for at least 2 hours. Swirl every 30 minutes to ensure the melted agar is evenly mixed and cooled.  
*The 42°C temperature is critical, too hot of a temperature will kill the DH10B cells, and too cool and the agar will set.*
- 5 Remove thin LB+agar plates from 4°C and keep at 37°C until ready to pour the top agar (minimum 30 minutes).
- 6 Thoroughly clean laminar flow as described below for phage prevention. Lay out the thin agar plates and remove the lids.
- 7 To one 500ml bottle of melted top agar, add 11.25 ml of overnight culture of DH10B. Mix gently, but thoroughly, by gently rocking and rolling the bottle, without introducing air bubbles. (Reduce the volume of culture added to smaller volumes of top agar as follows: (100 ml + 2.25 ml; 200 ml + 4.5 ml; 300 ml + 6.75 ml; 400 ml + 9.0 ml).
- 8 Pour a layer of seeded top agar onto the set LB agar plates. Do not flame the plates as this will kill the DH10B cells. Use a sterile pipette tip to push any air bubbles to the side of the plate.
- 9 Allow the plates to cool and set thoroughly before replacing the lids.
- 10 Repeat steps 7 to 9 with all bottles of top agar.

11 Once the phage assay plates are cool and set, they can be used to stamp or streak out the BAC clones. In most cases, the clones are transferred with a 96-pin tool (V&P Scientific) from the 96 well glycerol stock plates to the top agar. Incubate the plates, NOT INVERTED, at 37°C overnight, sealed in a saran wrap and parafilm.

**Result:** The seeded DH10B in the top agar will grow overnight causing the top agar layer to become opaque. If phage is present in the sample being tested, it will infect the DH10B resulting in a clear plaque in the DH10B lawn (Figure 3).



**Figure 3:** Phage assay plate demonstrating lysis of top agar bacterial lawn after 18 hour incubation with phage infected BAC clone cultures: A) non-lytic BAC colony, B) air bubble artifact, C) non-lytic BAC plaque (overgrowth), D) clear plaque from lytic BAC colony.

**Notes:** Plates must be used on the day they are made or discarded.

1) It is possible to pour the thin LB agar plates in advance, and store at 4°C. They must be warmed to 37°C before having the top agar poured onto them.

2) Left over culture of DH10B must be decontaminated - add 10ml of 10% Bleach, mix thoroughly, leave for at least 15 minutes, then discard in a sink with plenty of running water.

3) Top agar bottles must also be decontaminated – spray 10% bleach, fill the bottle with hot water, shake well and leave for at least 15 minutes. Flush down the sink with plenty of running water.