# Microbubbling technique

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## Abstract

For use in the protocol "Large Volume Marine Cyanophage protocols"

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## Protocol

**Step 1.**
Inoculate 2L Pro99 with exponentially growing cells (20-50 ml of dense cells, $10^8$ cells $ml^{-1}$)

**Step 2.**
Let cells grow to about mid $10^7$ cells $ml^{-1}$

**Step 3.**
Add as much cyanophage stock as available (up to an infective MOI = 3)

NOTES
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NOTE: Average cyanophage lysates are ~10^8 - 10^9 SYBR ml^-1; only ~0.1-10% of that is usually infective (as assayed with MPNs)

**Step 4.**
Adsorb for about 60 minutes without bubbling

DURATION
01:00:00

**Step 5.**
Start bubbling again

**Step 6.**
Add 1xPro99 amounts of N, P every 3 days until see 'lysis' (by eye, or more rigorously by a decrease in cell concentration)

**Step 7.**
Confirm phage production by SYBR titering