Quick Guide:

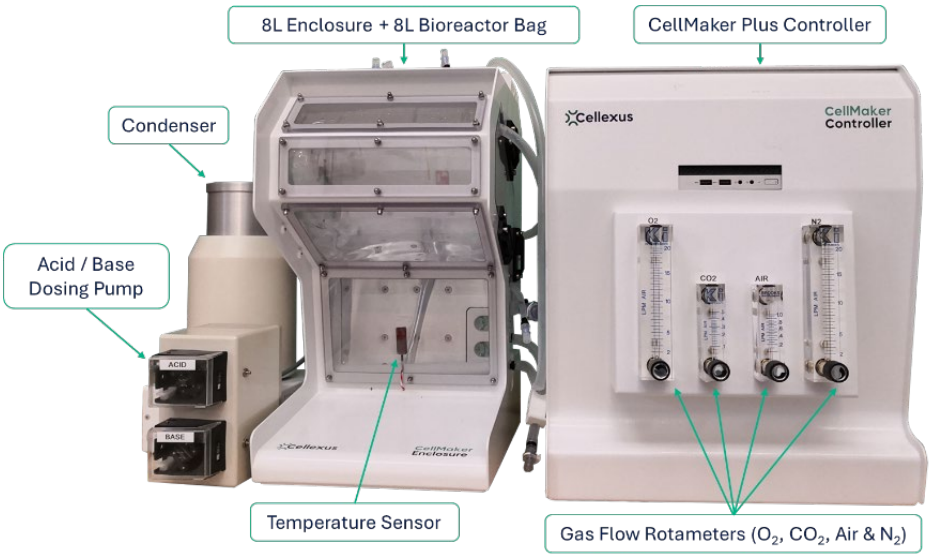
Bacteriophage Amplification with CellMaker

Introduction

Bacterial infections are most commonly treated with antimicrobials; however, the overuse and misuse of these drugs have led to the emergence of Antimicrobial Resistance (AMR)—a phenomenon where bacteria develop mechanisms to survive treatment. AMR is an escalating global threat to human, animal, and plant health, as well as to the environment. It is projected to contribute to up to 10 million deaths annually by 2050. In 2021 alone, an estimated 4.71 million deaths were associated with AMR, with 1.14 million deaths directly attributed to it (Cellexus, 2025; Ali et.al, 2020).

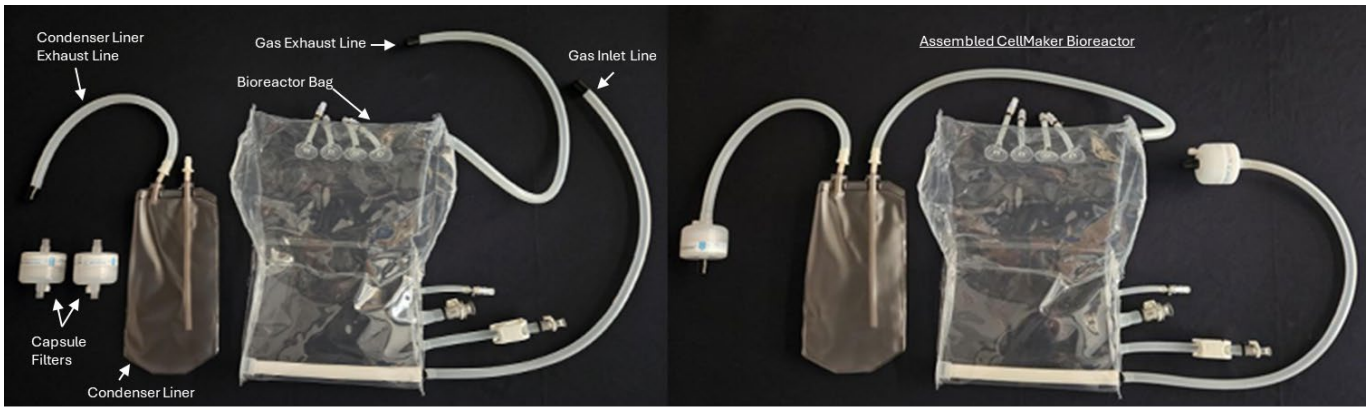
Bacteriophages—viruses that specifically infect and kill bacteria—are gaining attention as a promising alternative to conventional antibiotics for the prevention and treatment of bacterial infections. Unlike broad-spectrum antibiotics that target structures common to many bacterial species (and often kill beneficial bacteria in the process), bacteriophages are highly specific and can be tailored to target only harmful pathogens. Moreover, because they co-evolve with bacteria, phages can adapt to overcome emerging resistance mechanisms, making them a dynamic and sustainable solution to the growing AMR crisis.

The CellMaker bioreactor system offers an easy-to-use, scalable platform for efficient bacteriophage amplification. It provides flexible control over critical bioprocess parameters, including air flow for agitation, temperature regulation, and optional monitoring of dissolved oxygen (DO), pH, and pressure. The system uses sterile, single-use bioreactor bags to prevent batch-to-batch contamination. With multiple integrated ports for easy sampling, inoculation, and media transfer CellMaker streamlines the phage production process enabling phage therapies to be produced faster and with lower complexity and cost than is otherwise possible.



This quick guide provides insight into the available options for optimising your phage amplification process. These are intended as recommendations rather than strict protocols, and should be adapted to suit your specific strain and process needs. Included is data supplied by Proteon Pharmaceuticals, who are producing bacteriophage cocktails to prevent *Salmonella* infections in farm animals, demonstrating the real-world application of the CellMaker in commercial phage production.

Bioreactor Assembly



To preserve sterility of the bioreactor, remove the four components parts from the sterile packaging within a laminar flow hood using good aseptic technique. Within the flow hood connect the bioreactor bag gas exhaust line to the inlet of the Condenser Liner. Next, fit the capsule filters to the Condenser Liner exhaust line and the Gas Inlet Line to create a closed system.

Then install any immersion probes (e.g. pH, optical density probe) that are being used and the media fill/harvest tubing.

Once assembled the bioreactor can be removed from the flow hood/chamber and fitted into the CellMaker Enclosure.

Gas Connections

Gasses are supplied to the CellMaker Controller via standard 8 mm push-fit nylon gas lines from any supply source which has been regulated to a 2.5-5 bar pressure.

		Integrated compressor	Gas Inputs		
			Aux	2	3
CellMaker Regular	**Typical Gas**	Atmospheric air	O_2	N/A	N/A
	Control	Software Set-point (lpm)	Software Set-point (lpm)	N/A	N/A
CellMaker Plus	**Typical Gas**	Atmospheric air	O_2	N_2	CO_2
	Control	Software Set-point (lpm)	Software Set-point (lpm) & Automatic (DO%)	Manual Set-point (lpm)	Manual Set-point (lpm)

NB: Whilst the above indicates the typical gasses used for the Gas Inputs these can be interchanged and exchanged as required by the protocol being run. CellMaker is compatible with any non-corrosive gasses.

Air is supplied via an integrated compressor within the CellMaker Controller unit.

The gasses are blended within the CellMaker controller and supplied to the CellMaker Bioreactor Gas Inlet Line via a 0.22 µm capsule filter to maintain sterility of the culture.

The Bioreactor's exhaust gas passes through a cooled condenser unit to remove moisture and ultimately through a 0.22 µm capsule filter before venting to the environment, into a fume cabinet or extractor system. In the case of anaerobic experiments which are venting into an environment containing normal atmosphere it is recommended that a one-way valve be incorporated within the exhaust line to preventing atmospheric air from re-entering the system.

Cell Culture Procedure

Step 1 – Media Filling and Initial Setup

Fill the bioreactor bag with sterile media, ideally pre-heated to the desired optimal temperature for the microbial strain via the media fill/harvest tubing using a peristaltic pump. Set the desired temperature and gas flow rate. Press “Run” to initiate air supply and allow the system conditions to stabilise (~5-10 minutes).

Step 2 – Dissolved Oxygen (DO) Sensor Calibration (Ignore if not relevant)

Once gas flow and internal pressure has stabilised (0.1 mbar fluctuation normal and expected), perform a 3-point DO calibration using the CellMaker software.

Step 3 – Culture

Prior to microbe inoculation, add any supplementary nutrients or additives via the sampling port. Inoculate the bioreactor with the desired microbe via the sampling port. The cells can then be left to cultivate at the defined parameters and their growth monitored via either real-time monitoring (Optura Spy) or off-line optical density (OD_{600}) measurements.

Step 4 – Bacteriophage Infection

Once the inoculated bacterial culture has reached the desired optical density (OD_{600}) for infection—typically within the early logarithmic growth phase—the bacteriophage is introduced into the bioreactor via the sample port.

Initiating infection at an OD_{600} between 0.2 and 0.4 may be the optimal, as this range balances sufficient host cell density for effective phage propagation while avoiding overcrowding that can limit phage-host interactions. Although variations may exist between different phage-host systems, an OD_{600} of approximately 0.25 has been proposed as a standardised infection point for *E. coli* and *S. aureus* (Ali et. Al., 2020).

Step 5 – Harvesting

Following infection, the culture is maintained under optimal conditions to allow the bacteriophages to replicate until the desired titre (measured in PFU/mL) is achieved. Once amplification is complete, the phage-rich suspension is harvested through the designated media/harvest tubing port ready for downstream processing, such as filtration, purification, and concentration, depending on the intended application.

Case study Results Snapshot

An unoptimised comparative trial was conducted to evaluate bacteriophage amplification on *Salmonella enterica* serovar Enteritidis culture.

The CellMaker Bioreactor was filled with 5L of LB cultivation media and pre-warmed to 37°C. Then media was inoculated with overnight culture of S. enterica Enteritidis strain. The bioreactor was supplied continuously by 5L of air per minute and the temperature was stable at 37°C. Optical density of growing bacteria was measured every half an hour. When density reached OD600 0.4-0.5 (2h after bacterial inoculation) the inoculum with bacteriophages, specific for Salmonella, in amount of 5x107PFU (Plaque Forming Units) was added. Then the culture was sustained for another 3h to let the bacteriophages amplify. Samples were taken to measure titre of bacteriophages (in PFU/ml) and optical density of bacteria. Another process in the same conditions but without bacteriophages was conducted as a control to compare the growth of bacteria

Table 1: Experimental parameters

	Control	Phage production
Media	**LB Broth**	**LB Broth**
Media volume	**5L**	**5L**
Bioreactor	**CellMaker, 8L Regular**	**CellMaker, 8L Regular**
Cells	***S. enterica***	***S. enterica***
Flow Rate	**1LPM (default air)**	**1LPM (default air)**
Temperature	**37°C**	**37°C**
Bacteriophage infection	**-**	**$5x10^7$ PFU at OD_{600} 0.4-0.5**

Table 2: Proteon Pharmaceutical Optical Density results at OD_{600} of *S. enterica* and Phage Titres at PFU/mL

Time	S. enteritidis growth control (OD_{600})	S. enteritidis growth with bacteriophages (OD_{600})	Titre of bacteriophages (PFU/ml)
0h	0.101	0.020	
30'	0.103	0.046	
1h	0.197	0.072	
1h30'	0.456	0.276	
2h	0.937	0.480	5.0×10^7
2h30'	1.960	0.933	
3h	3.020	1.290	6.8×10^8
3h30'	3.910	0.580	
4h	4.500	0.332	8.5×10^9
4h30'	4.620	0.356	
5h	4.700	0.440	$1.0x\ 10^{10}$

NOTE: Following the control experiment the number of S. enteritidis cells used to inoculate was reduced to aid the timing of phage infection

Summary

The CellMaker system offers a highly effective and efficient platform for bacteriophage production. By employing single use airlift bioreactors CellMaker is able to achieve high phage titres in volumes from 1.5 to 50 litres without the requirement for extensive validation of bioreactor cleaning methods. CellMaker is a plug-and-play bioreactor which enables users to readily swap between different size bioreactors to rapidly react to production requirements.

References

Ali, J., Rafiq, Q. and Ratcliffe, E. (2020) *Improving phage titre through examining point of infection* [Preprint]. doi:10.21203/rs.3.rs-17640/v1.

'Rise of the Superbugs' (2025b) *Cellexus*, 31 March. Available at: https://cellexus.com/blog/rise-of-the-superbugs/.#

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