Cellexus

Application Note:

Bacteriophage amplification results using the CellMaker

One of the main applications of the CellMaker bioreactor system is bacteriophage amplification. We have been working with numerous companies and below we have detailed some examples of our successful work with phages.

Example 1

Cellexus carried out a demonstration at a clinical-stage company providing cost-effective cures for bacterial infections. As pH and dissolved oxygen monitoring were not points of interest, the 8L Regular CellMaker was used to look at amplification of the company's proprietary phages.
Four litres of sterile TSB broth was pumped into the bioreactor, and 20 mL *E. coli* was inoculated after temperature was achieved.

Temperature: 37°C
Airflow: 5L/min

Time (mins)	OD
0	0.010
30	0.025
60	0.084
75	0.153
85	**0.227**
105	0.568

Phages were introduced after 85 minutes (in green), although the growth phase continued up to 105 minutes. After this point, the OD sharply declined and the solution went clear. The product was obtained by ultra-centrifugation and filtering through a 0.2 micron filter, and the final phage concentration was 2 x 10^{10} PFU per mL.

These are promising initial results for the first trial and are likely to be further improved with the addition of antifoam and optimisation to yield even better titres.

The CellMaker offers a fast and low-cost way to effectively manufacture phages for this biotech company.

Example 2

Cellexus provided a demonstration at a biotech company that develops CRISPR engineered antibacterial products to see how well the CellMaker could amplify their proprietary phages. 3.75L of LB media was sterilised and pumped into an 8L Regular CellMaker with antifoam. *E. coli* was then inoculated and grown for 120 minutes.

Temperature: 37°C
Airflow: 5L/min

Time (mins)	OD
5	0.01
30	0.01
60	0.05
90	0.19
120	**0.60**

Phages were introduced at 120 minutes (in green) at a titre of 1x10^{9} PFU/mL and the run was sustained for 4 hours. The final concentration was recorded at 3.0 x 10^{10} PFU/mL. At 30 times more than the starting titre, this was the highest amplification that the company has ever achieved with this phage and can be even further improved with process development.
In conclusion, the route to market in this instance is substantially reduced due to rapid *E. coli* growth and concentrated phage production.

Cellexus

Application Note:

Bacteriophage amplification results using the CellMaker

Example 3

A North American biotech company ran two different fermentations on the CellMaker 8L Regular to look at amplification of their proprietary phages. *Pseudomonas aeruginosa* was inoculated in the first run, and *Staphylococcus aureus* was inoculated in the second run. For both experiments, 4L of sterilised TSB broth was used and antifoam was added.

Temperature: 37°C
Airflow: 5L/min
Phage infection shown in green.

The first run gave a final PFU of 1.5 x 10^{10} per mL. The run also gave the same kinetics as a 100L batch already in production at the company—exemplifying the scalability of the CellMaker.

The second run was re-infected with phages after 225 minutes as the first infection was introduced at an insufficient titre. After clarification, the final concentration of the second run was 2.5 x 10^{7} PFU/mL and also gave the same kinetics as a 150L batch already in production at the company.

In conclusion, the initial results were encouraging and can certainly be improved upon with relevant optimisation. The parallels in kinetics to larger production batches display the capability of scalable processes with the CellMaker.

Run 1: Pseudomonas Aeruginosa

Time (mins)	OD
0	0.050
30	0.080
60	0.140
75	**0.220**
105	0.400
135	0.500
165	0.400
195	0.370
225	0.250
255	0.100

Run 2: Staphylococcus aureus

Time (mins)	OD
0	0.001
90	0.070
120	0.160
135	**0.230**
165	0.480
195	0.710
225	**1.27**
285	0.53
315	0.12

Contact details

Cellexus International Limited
6 Riverside Court, Mayo Avenue
Dundee, DD2 1XD, UK

Email: enquiries@cellexus.com
Office: +44 (0) 1382 666357
www.cellexus.com

Contact us for more information or to request a demonstration.