CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Supplementary Material

The EuLV® System, an inducible stable producer cell line for lentiviral vector production

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SUPPLEMENTARY DATA 1
LEAK TEST OF EULV PRODUCER CELL

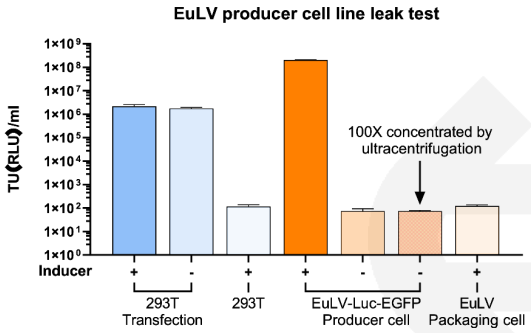


Figure 1

Figure 1 indicates the results of viral titer from EuLV® producer cell line (GOI: hPGK-luciferase-IRES-EGFP) without induction (leak titer). "+" indicates culture condition with an inducer, and "-" indicates culture condition without inducer. 293T cultured in FS293 medium and transfected with 19BF081 and packaging plasmids work as a control. The EuLV-luciferase producer cell line is cultured and induced for viral production as described previously (12E6 cell/mL with Feed). 293T and EuLV® packaging cell lines do not have the luciferase gene and work as background control. This data indicates EuLV® producer cell line has a 1,000,000-fold induction/non-induction ratio (orange bar). And the viral titer from the non-induction sample shows no obvious difference compared to the background of luciferase assay. Even after 100-fold concentration by ultracentrifugation (indicated by arrow), no obvious viral titer can be detected.

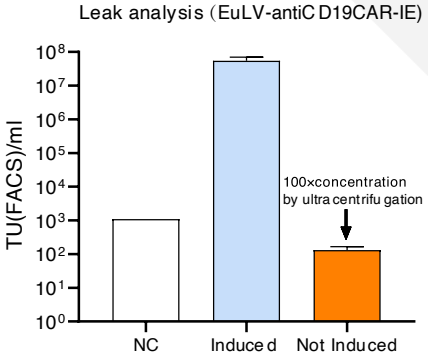


Figure 2

Figure 2 The packaging cell line without GOI worked as the negative control shown as this white bar. The blue bar showed the virus titer after induction. And the orange bar shows the titer of the non-induced sample after 100 times concentration by ultracentrifugation.

SUPPLEMENTARY DATA 2
EUBIOX

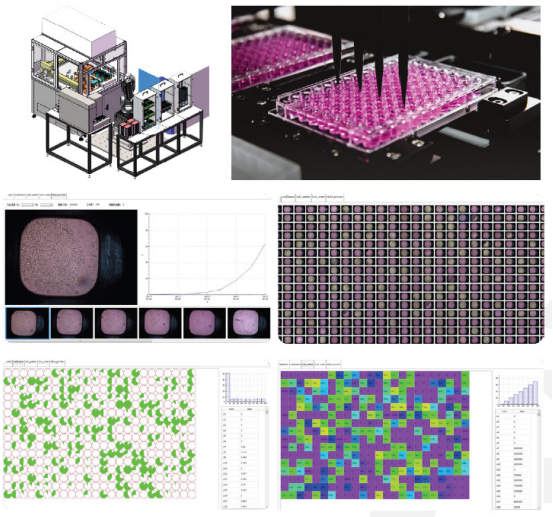
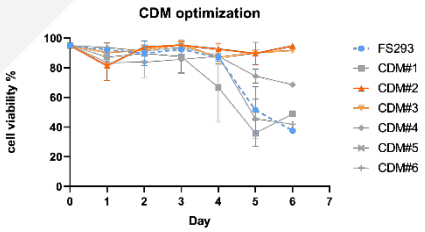


Figure 3

Figure 3 To increase the scale and accuracy of the screening, EurekaBio builds its automation facility, EuBioX. It could perform the routine operation of cell culture, monoclonal cell screening, cell-based bioassay, viral titer quantitation, DoE material preparation, and it has adapted to 384 to 6-well plates. With this, we can screen over 10,000 individual cell clones for each experiment round. EuBioX Non-Staining Live Cell Analysis System, a part of the automation system, can take a photo of a 384-well plate in less than 5 minutes. The EuBioX platform can record, trace, analysis, and pick the monoclonal with the best performance.

SUPPLEMENTARY DATA 3
MEDIUM OPTIMIZATION



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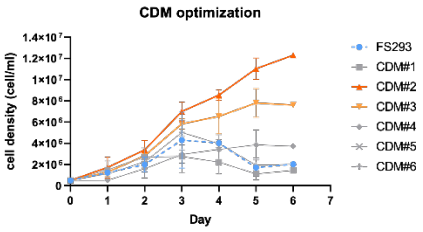

Figure 4

Figure 4 indicates the results of chemically defined medium (CDM) screening. 6 types of CDMs are tested, and the FreeStyle293 medium (FS293, Gibco) is used as a positive control. The two figures are the cell density and viability data during 6 days of suspension culture. CDM#2 and CDM#3 can support cell density higher than 6E6 cell/mL with viability above 90% after 6 days of continuous culture.

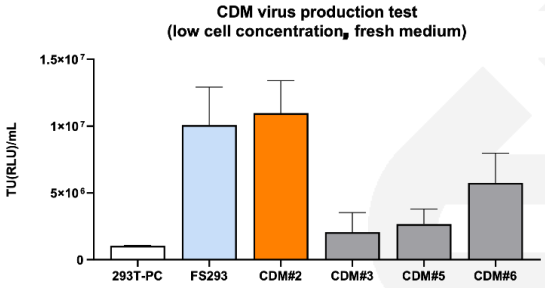

Figure 5

Figure 5 In CDM#2, cell density can reach as high as 1.2E7 cell/ mL. When tested for lentiviral production (luciferase titer), only CDM#2 can produce viruses with titer comparable to FS293 medium. This experiment is performed with EuLV® producer cell line cultured in low cell density (4E6/mL), and the culture medium is replaced just before induction. 293T-PC shows the titer data from transient transfection of suspension-cultured 293T cell, working as a positive control. CDM#2 is used for further process development.

SUPPLEMENTARY DATA 4

OPTIMIZATION OF LENTIVIRAL VECTOR PRODUCTION METHODS

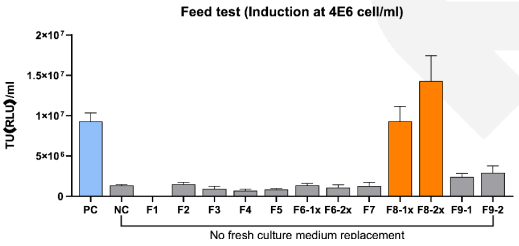

Figure 6

Figure 6 Feed screening results for F1 to F9, when the cells are induced for viral production after reaching a density of 4E6 cell/mL. Supplement F8-2x can support viral production in this cell density without the need for medium replacement. Cells cultured in the same condition but with 100% medium exchange just before induction works as a positive control (the blue bar).

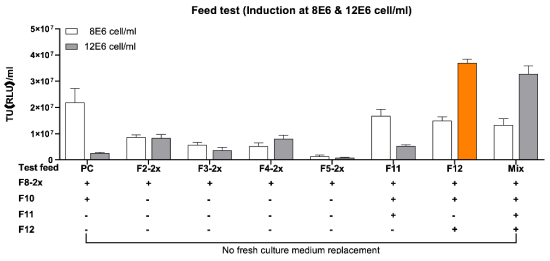

Figure 7

Figure 7 When the cell density of induction further increases to 12E6 cell/mL, an additional F12 supplement is required. The final feed formulation is composed of F8-2x, F10, and F12, and all these three supplements are homemade which are chemically defined. With this feed, EuLV® producer cells can grow and be induced for viral production at the cell density as high as 1.2E7 cell/mL in a feed-batch process without any medium replacement.

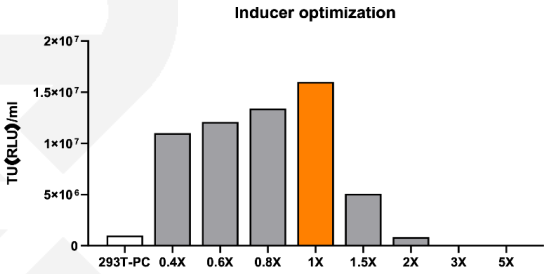

Figure 8

Figure 8 Effects of different concentrations of inducers on lentiviral production, 293T-PC represents lentiviral titer results from transient transfection of 4 plasmids with 293T cells.

SUPPLEMENTARY DATA 5

LENTIVIRAL PURITY PROFILE

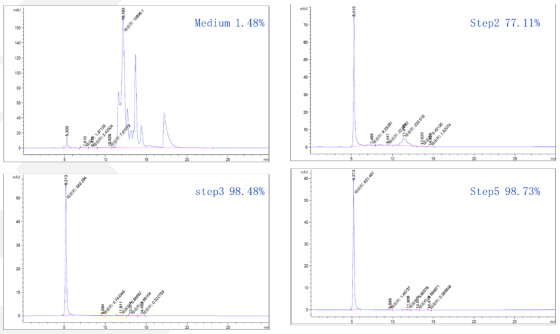

Figure 9

Figure 9 Lentivirus purity data analyzed by HPLC-SEC column after several purification steps. The peak time of lentivirus was 5.2 minutes, and the virus purity in the harvested medium was 1.48%. After 5 purifications, the virus purity could reach 98%.

DISCLAIMER

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