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Supplemental information

Packaging cells for lentiviral vectors generated using the cumate and coumermycin gene induction systems and nanowell single-cell cloning

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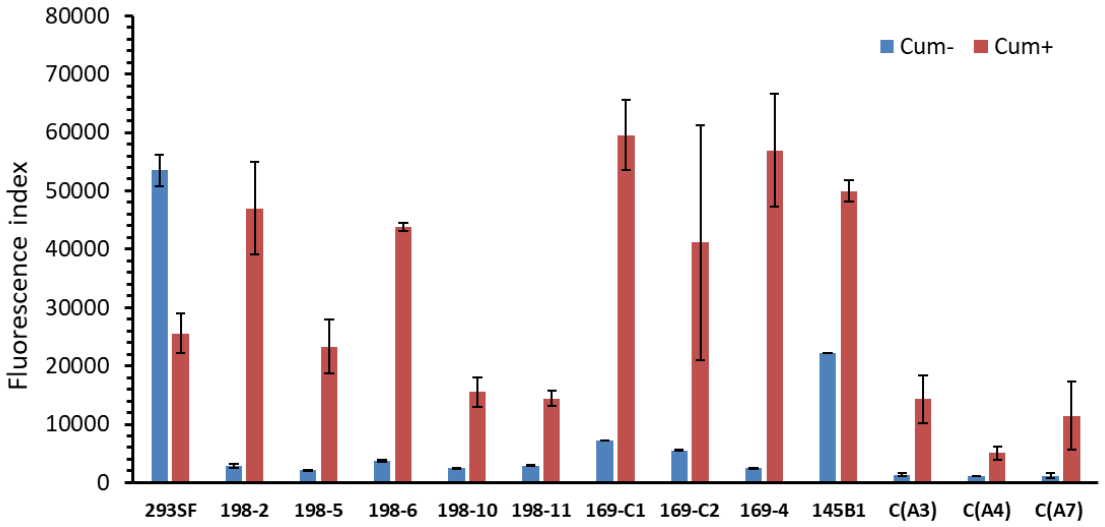

Figure S1: Induction analysis of clones expressing the CymR repressor.

Cells from different clones of 293SF-CymR (198-2, 198-5, 198-6, 198-10, 198-11, 169-C1, 169-C2, 169-4, 145B1, C(A3), C(A4), C(A7)) were transduced with a lentivirus expressing GFP regulated by the cumate inducible promoter (LV-CMV5CuO-GFP). The cells were induced (Cum+) or not (Cum-) the next day by addition of cumate and analyzed by flow cytometry for GFP expression two days later (three days post-transduction). 293SF are the parental cell line without the CymR (293SF-3F6 cells). The experiment was performed in duplicate. The data are mean ± SD.

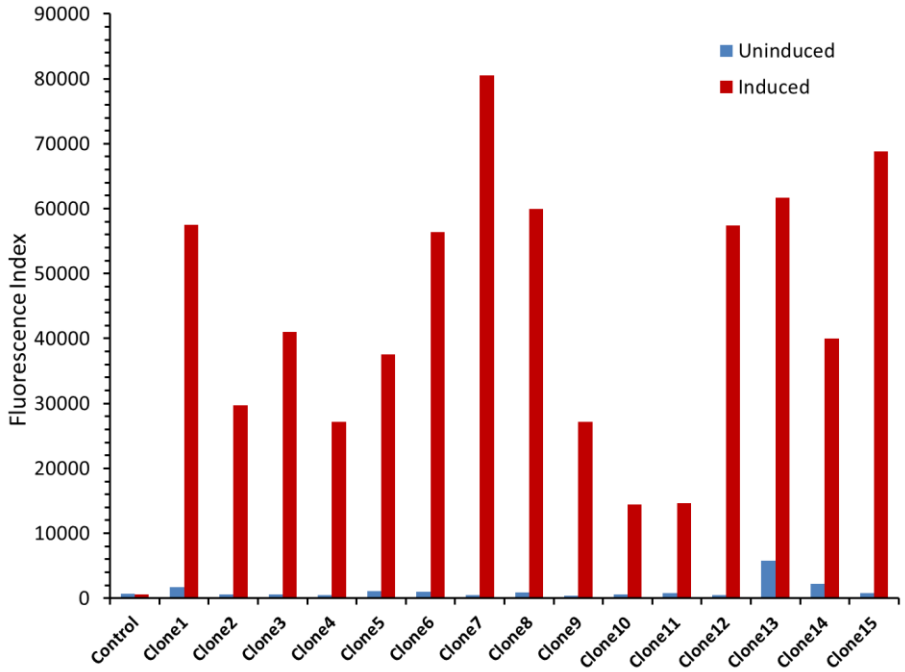

Figure S2: Induction analysis of clones expressing CymR and λR-GyrB during screening.

Cells from different clones of 293SF-CymR/GyrB were transduced with LV-12xlambda-TPL-GFP. The next day, the cells were induced or not (Uninduced) by addition of cumate and coumermycin. GFP expression was analysed by flow cytometry two days later (three days post-transduction). Control is the parental cell line (293SF-CymR).

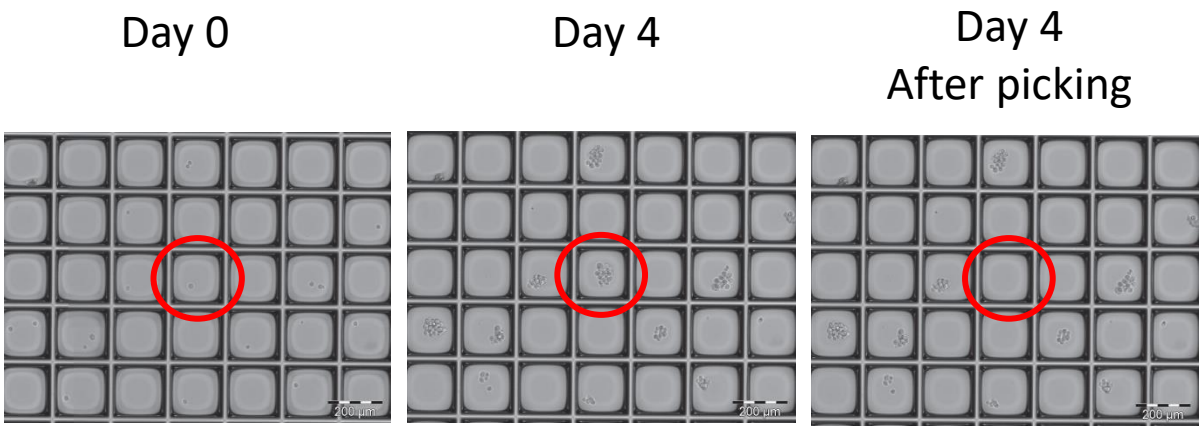

Figure S3: Documentation of monoclonality.

The nanowell array is scanned the day of plating (Day 0) to identify nanowells containing a single cell (red circle). The same nanowells are then scanned 3 or 4 days later before and after picking.

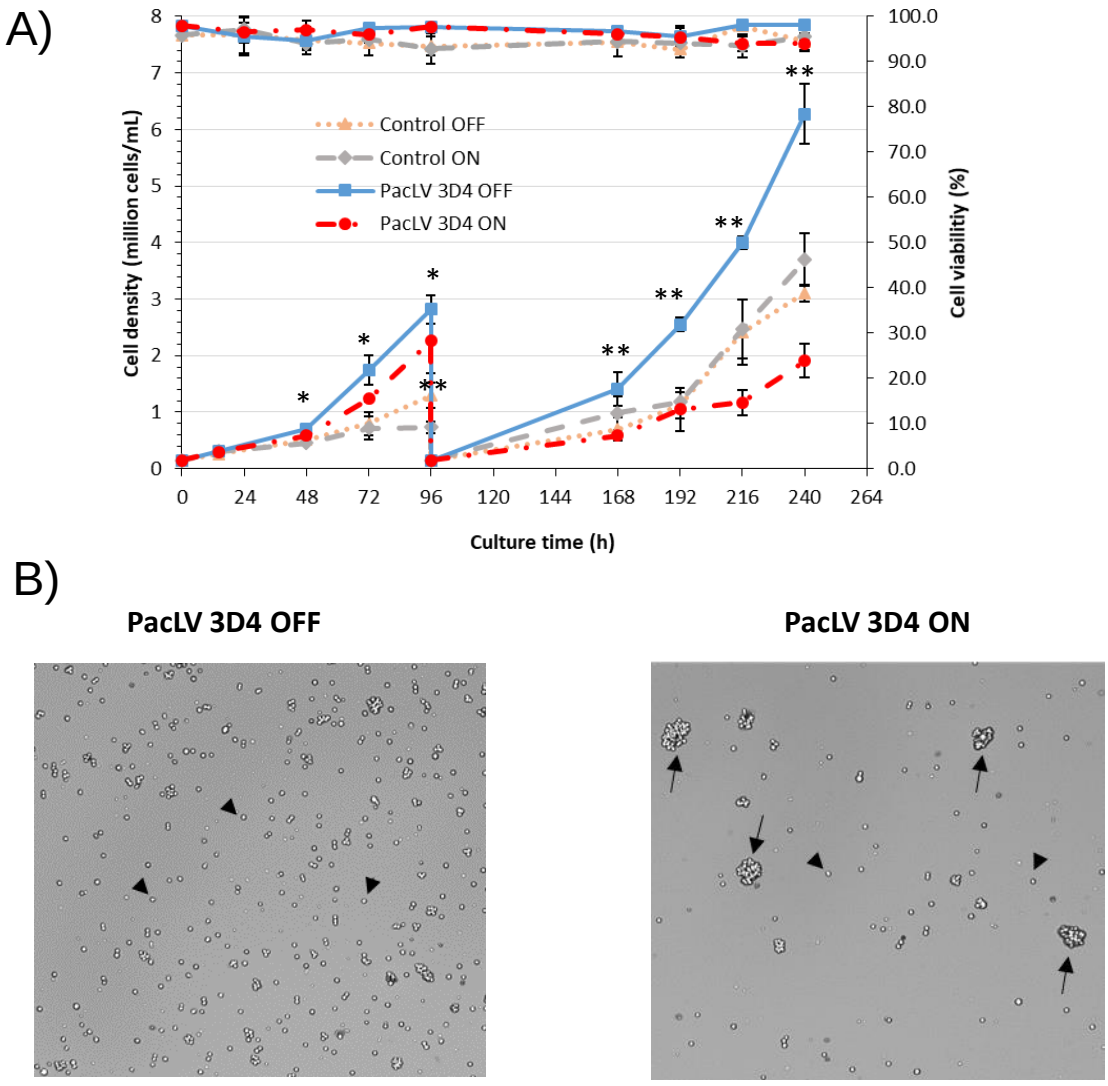

Figure S4: Effect of induction on growth of packaging cells.

Cells from packaging clone 3D4 were induced (PacLV 3D4 ON) or not (PacLV 3D4 OFF) with cumate and coumermycin and cultured in suspension for 10 days. As control, cells from the parental cell line (29SF-CymR/λR-GyrB) were cultured in parallel in the absence (Control OFF) or presence (Control ON) of cumate and coumermycin. **(A)** The cell density and viability were measured at the indicated time points. The cells were diluted to 150 000 cells per ml at 96 h. The experiment was performed in triplicate. The data are the mean ± SD. Note the packaging cells grow faster than the parental cell line. Induction significantly reduced the growth rate of the packaging cells from 48 h and thereafter (* = $P < 0.05$, ** = $P < 0.01$). There was not significant difference between induced and non induced parental cell line except at 96 h. **(B)** Pictures of packaging cells after 10 days of culture in the absence or presence of inducers. Arrowheads point to single cells and arrows point to cell aggregates.

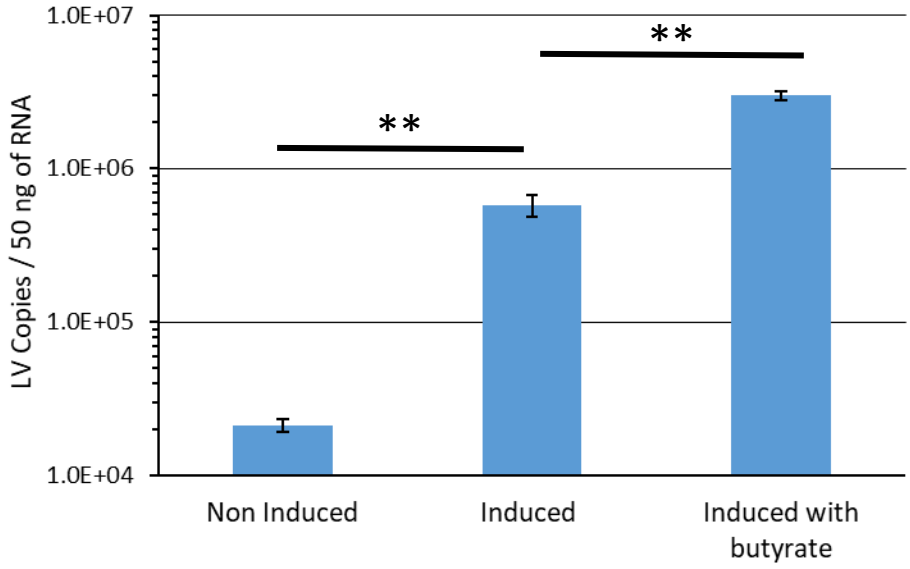

Figure S5: Effect of sodium butyrate on vector RNA production.

Cells from producer clone 3E9 were induced by addition of cumate and coumermycin. The next day, the cells were treated with sodium butyrate. The next day (48 h post-induction), cellular RNA was isolated and the amount of vector RNA was quantified by RT-PCR using primers specific for the LV packaging signal. Non induced are cells from clone 3E9 without cumate, coumermycin and sodium butyrate. The experiment was performed in triplicate. The data is the mean ± SD. **: value significantly different for each other ($P < 0.01$).

12xlambda-TPL

TCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGT
GATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTG
GCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTAC
CTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAG
TTTACCTCTGGCGGTGATAGTCGACTCTAGATAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGCTC
GTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGG
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TGAGGACAAACTCTTCGCGGTCTTTCCAGTACTCTTGGATCGGAAACCCGTCGGCCTCCGAACGGTACTC
CGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGATCGGAAAACCTCTCGAGAAAGGCGTCTAAC
CAGTCACAGTCGCAAGGTAGGCTGAGCACCGTGGCGGGCGGCAGCGGGTGGCGGTCGGGGTTGTTTCTG
GCGGAGGTGCTGCTGATGATGTAATTAAAGTAGGCGGTCTTGAGACGGCGGATGGTCGAGGTGAGGTGT
GGCAGGCTTGAGATCCAGCTGTTGGGGTGAGTACTCCCTCTCAAAAGCGGGCATTACTTCTGCGCTAAG
ATTGTCAGTTTCCAAAAACGAGGAGGATTTGATATTCACCTGGCCC

13xlambda-TPL

TCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGT
GATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTG
GCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTAC
CTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAGTTTACCTCTGGCGGTGATAGTCGAG
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TGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGTCACTCTCTTCCGCATCGCTGTCTGCGA
GGGCCAGCTGTTGGGCTCGCGGTTGAGGACAAACTCTTCGCGGTCTTTCCAGTACTCTTGGATCGGAAAC
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CCTCTCGAGAAAGGCGTCTAACCAGTCACAGTCGCAAGGTAGGCTGAGCACCGTGGCGGGCGGCAGCGG
GTGGCGGTCGGGGTTGTTTCTGGCGGAGGTGCTGCTGATGATGTAATTAAAGTAGGCGGTCTTGAGACG
GCGGATGGTCGAGGTGAGGTGTGGCAGGCTTGAGATCCAGCTGTTGGGGTGAGTACTCCCTCTCAAAAG
CGGGCATTACTTCTGCGCTAAGATTGTCAGTTTCCAAAAACGAGGAGGATTTGATATTCACCTGGCCC

Figure S6: Nuclotide sequences of 12xLambda-TPL and 13xLambda-TPL.

Table S1
Quantification of ghost wells after plating into nanowells

	Empty nanowells Day 0	Ghost nanowells Day 3	Probability of monoclonality
Test 1	5369	1	>99.9%
Test 2	3659	0	>99.9%
Test 3	1881	8	99.6%
Test 4	1885	2	99.9%
Test 5	3236	0	>99.9

The probability of monoclonality was calculated for each experiment by comparing the number of ghost wells observed at day 3 post plating with the total number of empty wells analyzed on the day of plating (day 0).
Tests 1 to 3: pools of GFP positive cells containing 8 to 15 % of DsRed positive cells were plated.
Test 4: a pool of 50% GFP and 50% DsRed cells was plated.
Test 5: pools of DsRed-positive and GFP-positive cells were plated in different macrowells