**Supporting Information**

# Viral generation, packaging, and transduction on a digital microfluidic platform

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## EXPERIMENTAL SECTION

### Reagents and materials

Microfluidic device fabrication reagents and supplies included chromium-coated glass slides with S1811 photoresist from Telic (Valencia, CA), indium tin oxide (ITO)-coated glass slides, RS =15-25Ω (cat no. CG-61IN- S207, Delta Technologies, Loveland CO), MF-321 positive photoresist developer from Rohm and Haas (Marlborough, MA), CR-4 chromium etchant from OM Group (Cleveland, OH), AZ-300T photoresist stripper from AZ Electronic Materials (Somerville, NJ), DuPont AF from DuPont Fluoroproducts (Wilmington, DE). Transparency masks for device fabrication were printed from CAD/Art (Bandon, OR). General chemicals for tissue culture were purchased from Wisent Bio Products (Saint-Bruno, QC, Canada). Invitrogen Lipofectamine 3000 Transfection Reagent was purchased from Thermo Fisher Scientific (Waltham, MA). Unless specified otherwise, general-use chemicals and kits were purchased from Thermo Fisher Scientic and Sigma-Aldrich (St. Louis, MO). Plasmids for this study were purchased from Addgene or donated (**Figure S1**, **Table S1-S2**) and primers (**Table S3**) were purchased from Invitrogen (Waltham, MA).

### Device fabrication and assembly

Digital microfluidic devices were fabricated following methods as introduced previously[1]. Briefly, designs were drawn using AutoCAD 2018 (Autodesk, San Rafael, CA) and photomasks were then printed in high-resolution (20,000 dpi) by CAD/Art Services Inc (Bandon, OR). The bottom-plates bearing patterned electrodes were formed by standard photolithography techniques. Chromium substrates coated with photoresist

were UV-exposed through the photomask (7s, 42.4 mW/cm$^2$) to imprint the transparency mask designs. Substrates were then developed in MF-321 positive photoresist developer (2 min, shaking), rinsed with DI water, dried under a stream of nitrogen and baked for 1 min at 115°C. The exposed chromium was then etched using CR-4 chromium etchant (3 min) and substrates were then rinsed with distilled water and dried under a stream of nitrogen. Finally, devices were immersed in AZ300T photoresist stripper (3 min) to remove any remaining photoresist before being rinsed and dried under a stream of nitrogen. Once the patterning step was completed, the substrates were immersed in a silane solution consisting of deionized water, isopropanol and 3-(Trimethoxysilyl)propyl-methacrylate (50:50:1) for dielectric priming during 15 min. Substrates were rinsed with isopropanol, distilled water and then dried under a stream of nitrogen. Prior to the addition of the polymer coating to complete the process, thermal tape was added on top of the contact pads to facilitate later removal of the polymer coating from the contact pads and allow electrical contact for droplet actuation. Parylene-C was used as a dielectric which was deposited by chemical vapor deposition in a SCS Labcoter 2 PDS 2010 (Specialty Coating Systems, Indianapolis, IN) achieving a uniform thickness of 7 μm. 1% Teflon-AF 1600 in FC-40 was used as a hydrophobic coating and was spin-coated in a Laurell spin-coater at 1000 rpm for 30 s followed by post-baking on a hot-plate (165°C, 10 min).

The DMF top-plates consist of a continuous ground electrode formed from an indium tin oxide (ITO) coated glass substrate. For typical ground plates, ITOs were spin-coated with the 1% Teflon-AF 1600 using the same program as described in the bottom-plate fabrication procedure. ITOs that have an array of hydrophilic spots (i.e., circular regions of exposed ITO) for on-chip tissue culture were microfabricated using a Teflon-

liftoff procedure (following procedures described previously. ITOs were cleaned by immersion in an RCA solution comprising of distilled water, 28% aqueous ammonium hydroxide, 30% hydrogen peroxide (5:1:1 v/v/v) for 30 min at 80°C on a hotplate. After rinsing, drying and dehydrating (2 min at 95°C), the substrates were spin-coated with Shipley S1811 photoresist (10 s, 500 rpm, ACL=100 rpm and 60 s, 3000 rpm, ACL=500 rpm) and baked at 95°C for 2 min. Slides were cut to the desired size (i.e.: 50 x 75 mm) using a Cutter's Mate (Creator's Stained Glass, Victoria, BC) and vented under a stream of nitrogen. Substrates were exposed through the photomask with an array of 12, 1.2 mm diameter circular features and two ~1.6 mm diameter circular features (10 s, 42.4 mW/cm$^2$) and were developed in MF-321 (3 min). After rinsing, air-drying and dehydrating (1 min, 95°C), top-plates were then flood exposed (10 sec, 42.4 mW/cm$^2$), spin-coated with 1% Teflon-AF 1600 (10 s, 500 rpm, ACL = 100 rpm and 60 s, 3000 rpm, ACL = 500), and post-baked on a hotplate (165°C, 10 min). After allowing to cool on aluminum foil for 2 min, substrates were immersed in acetone with gentle agitation for 10-15 s until the Teflon-AF over the patterned sites was lifted off. After being rinsed with DI water and dried under a stream of nitrogen, the substrates were post-baked to reflow the Teflon-AF at 165°C, 210°C and 300°C for 5 min at each temperature.

Complete devices were assembled with the continuous ground ITO top-plate and the chromium electrode-bearing bottom plate, being joined by stacking two layers of double-sided tape to a gap height of approximately 140 μm. Alignment of the ITO top plate above the bottom plate was performed with care such that smaller circular features aligned with the target cell culture regions and the larger circular features aligned with the

HEK293T cells culture regions (see **Figure 1** in main text). Mineral oil was coated on the electrodes by actuating a water droplet enveloped in mineral oil across the device.

**Automation setup and device operation**

The automation system[1] consists of a graphical user interface run by a Python program that is used to control an Arduino Uno microcontroller. A driving potential of 2.5 $V_{P-P}$ or ~500 V total was generated by amplification of a sine wave output from a function generator (Agilent Technologies, Santa Clara, CA) operating at 15 kHz by a PZD-700A amplifier, (Trek Inc., Lockport, NY) and delivered to the PCB control board. The Arduino controls the state of high-voltage relays (AQW216 Panasonic, Digikey, Winnipeg, MB) that are soldered onto the PCB control board. The logic state of an individual solid-state switch is controlled through an $I^2C$ communication protocol by an I/O expander (Maxim 7300, Digikey, Winnipeg, MB). This control board is mated to a pogo pin interface (104 pins), where each switch delivers a high-voltage potential (or ground) signal to a contact pad on the DMF device. See our GitHub registry (https://github.com/shihmicrolab/Automation) to assemble the hardware and to install the open-source software program to execute the automation system.

On-chip experiments were done through reagent loading by pipetting a droplet of liquid onto the outer-edge of a reservoir electrode and adjacent to the gap between the bottom and top plates and actuating the reservoir electrode. This will allow the droplet to slip under the top plate and be 'sandwiched' between the two plates. Once inside the reservoirs, the droplets were then actively dispensed, moved, mixed, or merged by sequential actuation of adjacent electrodes on the bottom plates. Active dispensing was

achieved over three electrodes and results in a droplet with a diameter of the same size as the electrodes (i.e. a unit droplet). To initiate passive dispensing, it is achieved by moving an actively dispensed droplet over the vacant lift-off hydrophilic spot. At times, contents on this spot may be displaced with the contents of a new source droplet. Generally, all droplets containing proteins were supplemented with 0.05% Pluronics F-127 (ThermoFisher). Waste and unused fluids were removed by delivering them to waste reservoirs and removed using KimWipes (Kimberly-Clark, Irving, TX).

**Lentiviral vector digestion, oligo annealing, and cloning**

*Digestion:* For lentiviral transfer plasmids, we followed a similar protocol described from the Zhang lab.[2-3] Briefly, 20 bp sgRNA scaffold were synthesized as oligonucleotides (ThermoFisher, Waltham, MA) after being designed via the Benchling online platform. The oligos were designed with Esp3I sticky ends (such that they can be ligated to the lentiCRISPR_v2 backbone (Addgene, plasmid #52961) (see **Table S1** for synthesized guides with sticky ends). The backbone was first digested using the Esp3I restriction enzyme by following the Thermofisher manufacturer's protocol (Waltham, MA). The digestion reaction consisted of 2 µL Anza$^{TM}$ 10X buffer, 1 µL Anza$^{TM}$ Esp3I enzyme, 2 µL DTT (10mM), 1 µg template DNA and topped to 10 µL final volume using di$H_2O$. The reaction tube was incubated at 37 °C for 15 min and immediately heat-inactivated at 65 °C for 20 min to avoid star-activity. The digested plasmid was run through a 0.8% agarose gel in TAE buffer at 100 V for 45 min and the backbone band (~13 kb) was gel purified using the GeneJET gel extraction kit (ThermoFisher, Waltham, MA).

*Annealing:* Each pair of oligos were then phosphorylated and annealed using Anza$^{TM}$ T4 PNK by following the manufacturer's protocol. The phosphorylating and annealing reaction consisted of 1 µL oligo 1 (100 µM), 1 µL oligo 2 (100 µM), 1 µL 10X T4 Anza$^{TM}$ ligation buffer, 0.5 µL Anza$^{TM}$ T4 PNK and 6.5 µL distilled water. Each reaction tube was put in the thermocycler using the following parameters: 37 °C for 30 min, 95 °C for 5 min and then ramping down to 25 °C at 5°C/min. The annealed oligos were then diluted with sterile water at 1:200 dilution.

*Cloning*: Following the annealing step, the annealed oligos were then ligated with the purified, digested lentiCRISPR_v2 backbone with the New England BioLabs T4 ligase (Whitby, ON) following the manufacturer's protocol. The ligation reaction consisted of 1 µL annealed oligo (gRNA), 50-100 ng backbone vector, 1 µL 10X T4 buffer, 1 µL T4 ligase and topped with nuclease-free water up to a final volume of 10 µL. The reaction tubes were incubated at room temperature for 10 min. The assembled products were transformed by heat-shock procedures (42 °C, 1 min; incubation on ice; 3 min) into a competent Stbl3 *E. coli* strain. The transformed products were grown on LB/ampicillin agar dishes and 3 colonies were picked for colony PCR as quick analysis for insert confirmation. Each colony that was picked was diluted in 20 µL of di$H_2O$. For the colony PCR procedure, one reaction consisted of 16.8 µL of Phusion high-fidelity mastermix buffer, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 1 µL of the diluted colony and 0.2 µL of the high-fidelity Phusion polymerase (Thermofisher, Waltham, MA). The following PCR thermocycling conditions were used: initial denaturation at 98 °C for 30 s followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s/kb, and a final extension step at

72 °C for 5 min. PCR products were loaded into a 0.8% agarose gel in TAE buffer and resolved at 130 V for 30 min. Positive clones were expected to yield a ~2 kb amplicon and negative clones were expected to yield no amplicons. The positive clones were then grown overnight liquid culture of 5 mL LB before being DNA purified. The constructed plasmids were verified by Sanger sequencing (Eurofins Genomics; Louisville, KY, USA).

To create a lentiviral plasmid with a fluorescent and antibiotic marker, we purchased pLentiCRISPR_mCherry was purchased (Addgene, plasmid #75161). We followed the manufacturer's protocol (New England BioLabs, Whitby, ON) for digestion and annealing to insert gene fragments of the antibiotic neomycin resistance that was PCR amplified from the plasmid mCherry2-N1 (Addgene, plasmid #54517) with flanking XbaI cut sites. The same cloning procedures were used, and this new assembly is thus named pLentiCRISPR_mCherry_NeoR to differentiate from the old one (see **Figure S11a**).

**New plasmid construction**

Since the pLentiCRISPR_mCherry backbone is derived from LentiCRISPRv1, which LentiCRISPRv2 is also derived from, the vector backbone digestion, oligo annealing, and cloning procedures for the sgRNA insert were the same as the previous section. However, due to perhaps the pLentiCRISPR_mCherry being compromised from the start, it was not possible to continue with pLentiCRISPR_mCherry_NeoR. Hence, part of LentiCRISPRv2 was amplified to keep the inserted sgRNA and every other element needed for the transfer lentiviral plasmid except for the Cas9 gene cassette and the puromycin antibiotic resistance (see **Figure S11b**). Part of pLentiCRISPR_mCherry_NeoR was amplified to keep the neomycin antibiotic resistance

gene cassette and the Cas9 gene linked to an mCherry fluorescent reporter. LentiCRISPRv2 and pLentiCRISPR_mCherry_NeoR were PCR amplified with Esp3I and EcoRI cut sites for correct orientation ligation using T4 ligase to produce pLentiCRISPR_mCherry_NeoRv2 (LCMNv2) (see Table S3). The assembled products were transformed into a competent Stbl3 *E. coli* strain, colony PCR validated for the sgRNA insert, grown overnight and sent for sequencing verification by Sanger sequencing (Eurofins Genomics).

**Macroscale cell culture, transfection, viral production and transduction**

*Cell culture:* H1299, HEK293T, MCF-7, MDA-MB-231, and T47D-KBLuc were grown in complete cell culture media formed from DMEM (4.5 g/L glucose, with L-glutamine, sodium pyruvate and phenol red) (HEK293T, MCF-7, MDA-MB-231) or RPMI 1640 with L-glutamine (H1299, T47D-KBLuc), supplemented with 10 % heat-inactivated fetal bovine serum. Cells were grown and maintained in 100 mm cell culture-treated petri dishes in an incubator at 37 °C with 5 % $CO_2$. For maintenance, cells were detached using a solution of trypsin-EDTA (0.25 % w/v) and resuspended in complete media and placed in a 100 mm petri dish. Once detached, the cells were centrifuged at 1,000 RPM for 5 minutes and the supernatant was aspirated. The cells were then resuspended in fresh complete medium and counted using a hemocytometer for specific cell density seeding.

*Transfection:* Liposome-mediated transfection followed protocols described previously[1]. 24-well plates were used for forward transfection experiments. Liposome-based transfection followed a 3-day timeline. Prior to transfection (day 0), cells were seeded at a density of 0.3 x $10^6$ cells/mL in a 6-well plate until it reached ~70 % confluency. On day 1, 2.5 μg of DNA was pre-mixed with 5 μL of P3000 reagent in 125 μL of Opti-MEM and

added to 11.25 µL of Lipofectamine 3000 reagent that was pre-mixed in 125 µL Opti-MEM. The lipid-DNA mixture was incubated at room temperature for 10 min, and then added to the cultured cells in the well-plate. On day 2 (after 24 h), the lipid-DNA complexes were removed from the wells by aspiration and fresh complete medium was replenished. Cells were stained with 1 µM Hoechst 33342 (Thermofisher, Waltham, MA, USA) (usually on day 3 or day 4) and incubated for 30 min for cell counting. Cells were imaged using a 20X objective on an Olympus IX73 inverted microscope (Olympus Canada, Mississauga, ON, Canada) that has fluorescence imaging capabilities (Hoechst: $\lambda_{ex}$ = 350 nm and $\lambda_{em}$ = 461 nm; GFP: $\lambda_{ex}$ = 488 nm and $\lambda_{em}$ = 509 nm; mCherry: $\lambda_{ex}$ = 585 nm and $\lambda_{em}$ = 608 nm). Fluorescence images were analyzed using an ImageJ pipeline.

*Lentiviral production:* To produce lentiviruses, two 10 cm petri dishes containing HEK293T cells were seeded at ~5 x $10^6$ cells/mL to achieve ~80% confluency. Once reached, the media was changed to fresh complete media and Lipofectamine 3000 transfection reagents were used. For each dish, 25 µL of Lipofectamine 3000 reagent was diluted in 475 µL of Opti-MEM medium with 1.5 µg of pMDLg/pRRE (Addgene, plasmid #12251), 1.5 µg of pRev (Addgene, plasmid #12253), 3 µg of pMD2.G (Addgene, plasmid #12259), and 6 µg of the transfer plasmid (pLv-mCherry - Addgene, plasmid #36084; non-target shRNA plasmid; ERα shRNA 1 (TRCN0000003300); LCMNv2_eGFP_12; LCMNv2_ESR1_76). Additional Opti-MEM medium was added to the mixture to top up the volume to 500 µL. The complete mixture was incubated for 20 min at room temperature before being added dropwise to the cells. After 24 h, the mixture in the dishes was removed and replaced with 6 mL of fresh complete media and incubated at 37 °C overnight.

After 24 h, the supernatant was collected in a 50 mL Falcon tube and was stored at 4°C until a second collection was made. Following another 24 h, a second collection of the supernatant was done, and the pH adjustment of the total supernatant volume was completed by adding HEPES (1.5 M, pH 7.5) in a 1:100 ratio. The supernatant was then filtered using a 0.45 μm polyethersulfone membrane filter (VWR, Mississauga, ON), and aliquoted into 1.5 mL Eppendorf tubes and stored at -80 °C.

*Counting titers:* HEK293T cells were seeded at a density of 4.0 x $10^4$ cells/mL in 100 μL DMEM supplemented with 10 % FBS in a 96-well plate and incubated overnight at 37 °C and 5% $CO_2$. Prior to titration, DMEM supplemented with 10% heat-inactivated FBS and 4 ug/mL of polybrene known as the "diluent" were prepared. Serial dilutions (three 1:10 serial dilutions followed by seven 1:3 serial dilutions) were prepared in a 96-well plate where each lentiviral titer was titrated in duplicates (**Figure S1b**). Each well containing HEK293T cells received 100 μL of the viral dilutions. After 24 h incubation, old viral media was discarded in bleach and 100 μL of fresh complete media without polybrene and containing 1 μg/mL of puromycin was added to each well and was refreshed each 2-3 days until 8-9 days. To estimate the transducing units (per mL), each well was washed twice with PBS and cells were stained with 0.1 % w/v crystal violet in 20% ethanol. After 10 min, the well plate was washed with water. Stained cells forming round colonies were counted and the transducing units per mL (TU/mL) were calculated as follows (**Equation 2**):

$$\frac{TU}{mL} = \frac{number\ of\ colonies}{total\ volume\ in\ well\ (mL)} \times final\ dilution\ factor$$

An alternative method for viral titration was used when a transfer plasmid did not contain a eukaryotic selection antibiotic resistance marker (e.g., pLv-mCherry). We followed a protocol similar to Drayman and Oppenheim[4] which relied on measuring the viral titer using flow cytometry. HEK293T cells were seeded on a 6-well plate at ~5 x $10^4$ cells per well to reach a confluency of 40-50% the following day. Prior to the transduction, while the lentiviral particles were thawing on ice, DMEM (with 10% heat-inactivated FBS) was supplemented with 8 µg/mL of polybrene to use as a diluent for several dilutions. Following the template as seen in **Table S4** lentiviral dilutions were made and applied to their corresponding well in the 6-well plate.

The plate was gently swirled by hand each 5 min for 30 min to ensure that all cells were coated with lentiviruses/media and returned to the incubator overnight with 5% $CO_2$ and at 37°C. 24 hours after the viral infection, 1.5 mL of normal complete media was added to each well and the cells were maintained for an additional two days until they were prepped for flow cytometry. For cell gating, each well was washed with 1 mL PBS and 150 µL of 0.25% trypsin-EDTA was used to detach the cells. The cells were collected in each their individual tube and washed with PBS again before centrifuging them at 1,000 RPM for 5 min. The supernatant was removed, and the cells were re-suspended in 3 mL of cell sorting buffer. The cells were then strained through a 40 µm cell strainer (VWR, Mississauga, ON), distributed into FACs sorting tubes (VWR, Mississauga, ON) and placed on ice until the BD FACs Melody (BD Biosciences) machine at Concordia's Genome Foundry was ready to be used. The cells were then gated for the percentage of mCherry positive cells. To determine the viral titer (TU/mL), **Equation 1** was used.

*Transduction:* For viral transduction of target cells, we followed a protocol similar to Li and Rossi which the company Sigma-Aldrich relied on for transducing MISSION® plasmid packaged lentiviral particles[5]. 1.6 x $10^4$ cells of target cells per well were seeded in a 96-well plate and then incubated overnight at 37°C and with 5% $CO_2$. After 24 h, the media was removed from the wells and replaced with 100 µL of media containing polybrene (8 µg/mL) for each well. Next, a determined volume of lentiviral particles of known titer was added to the appropriate wells. The plate was gently rocked by hand and then incubated overnight. The determined volume of lentiviral particles was calculated upon the multiplicity of infection (MOI) decided to use. The following **Equation 3** was used to know the volume of viral stock to use based on the MOI:

$$\frac{MOI \times total\ cell\ number}{Viral\ titer} = volume\ of\ viral\ stock\ to\ use \text{ (in mL)}$$

where MOI = TU/cell and viral titer = TU/mL

After 72 h, the medium containing lentiviral particles was removed and 100 µL of fresh media was added to each well. Images of the cells were taken with a fluorescent microscope and quantification was done with ImageJ.

*RNAi and CRISPR experiments:* Before transduction, target cells were seeded at a cell density of 1.6 x $10^4$ cells/mL in a 96-well plate. After 24 h, the cells were infected with shRNA-packaged or all-in-one Cas9 and sgRNA- packaged lentiviruses at an approximate MOIs of 0.5, 1, 2 and 10. Target cells were maintained for 7 days while refreshing the medium (RNAi: supplemented with 1 µg/mL of puromycin, CRISPR-Cas9: no antibiotics) every two days. After 7 days, the cells were collected by lysing them with 50 µL of DNase/lysis solution for qRT-PCR validation or lysing them with 52 µL of cell lysis buffer

(50 µL lysis buffer and 2 µL protein degrader solution) for genomic cleavage detection assays.

**qRT-PCR**

Gene-specific primers were designed on Benchling (**Table S5**) and ordered via Biocorps (Montréal, QC). Cell lysis was performed as described in the manufacturer's Cells-to-$C_T$™ 1-Step *Power*SYBR® Green kit protocol (ThermoFisher, Waltham, ON). Cell medium was removed, and the cells were washed with ice-cold PBS buffer. After aspiration of PBS, 50 µL of room-temperature DNase/lysis solution was added and mixed to each well. The well plate was incubated at room temperature for 5 mins and 5 µL of room-temperature stop solution was then added. The well plate was incubated for another 2 min at room temperature and then the lysates were placed on ice for the next step. The RT-PCR master mix was prepared on ice which consisted of 10 µL of Power qRT-PCR mix, 0.16 µL Power RT mix, 2 µL of the gene-specific forward and reverse primer (10 µM) and nuclease-free water up to 18 µL. The appropriate volume of qRT-PCR master mix and lysate were added to a 48-array plate. The reactions were run in the ECO real-time PCR machine (Illumina) at the following conditions: reverse transcription (1 cycle, 48°C, 30 min), polymerase activation (1 cycle, 95°C, 10 min), amplification (40 cycles; 95°C, 15 sec; 60°C, 1 min) and melt curve step (1 cycle; 95°C, 15 sec; 60°C, 15 sec; 95°C, 15 sec). Data produced from the qt-PCR reactions were further analyzed using the ECO software provided with the ECO system.

**Gene cleavage detection assay**

To validate CRISPR-Cas9 knock-outs, the gene cleavage detection assay was done with the GeneArt genomic cleavage detection kit (Thermofisher). After performing CRISPR assays, the cells were collected and lysed with 50 µL of the provided lysis buffer and 2 µL of protein degrader solution. Once lysed, the whole cell lysate mix was transferred into a PCR tube for the following thermocycler conditions: 68°C for 15 min, 95°C for 10 min and 4°C on hold. The reaction was then PCR amplified for the region of interest where the CRISPR-Cas9 cut has occurred. One PCR reaction consisted of 2 µL of cell lysate, 1 µL of forward and reverse primers mix (10 µM), 25 µL of provided AmpliTaq® Gold 360 Master mix and 25 µL of di$H_2O$. The following parameters were used for the thermocyler run: enzyme activation (1 cycle, 95°C, 10 min), 35 cycles of denaturation (95°C, 30 sec), annealing (57°C, 30 sec) and extension (72°C, 30 sec), a final extension (72°C, 7 min) and finally infinite hold at 4°C. The PCR product was then run on a 0.8% gel at 130 V for 30 min to verify that the correct amplicon was present. The cleavage assay was first set up by purifying the PCR product and then by denaturing and re-annealing the PCR fragments to form heterogenous DNA duplexed. Briefly, 200 ng of PCR product was combined with 1 µL 10X detection reaction buffer in a PCR tube. The volume was then brought to a total of 9 µL with water. The PCR tube was placed in a thermocycler for the following run: 95°C for 5 min, 95°C-85°C for a ramping of -2°C/sec, 85°C-25°C for a ramping of -0.1°C/sec and finally 4°C on hold. The next step consisted of cleaving the heteroduplex DNA containing the indel with the detection enzyme. For this, 1 µL of detection enzyme was added to the previous PCR tubes to have a total reaction volume of 10 µL. The reaction was incubated at 37°C for one hour and then was

immediately ran on a 2% gel using lithium acetate borate (LAB) buffer at 220 V for 20 min. Finally, the gel was scanned using a UV transilluminator and the imaging system ImageJ was used to determine the relative proportion of DNA contained in each band to calculate the cleavage efficiency (see **Equation 2** below).

$$Cleavage\ efficiency = 1 - [(1 - fraction\ cleaved)^{\frac{1}{2}}]$$

**Imaging pipeline on ImageJ**

Images that were taken via microscopy were acquired using a Hamamatsu digital camera (Model C1140-42U) with the HCImageLive software. The images were then processed using ImageJ using a protocol similar to previous work[1]. For each image, the channels were split, and their brightness and contrast were adjusted for clear identification of the cells against the background. The image was then duplicated for processing and the threshold was adjusted to turn the duplicated item into a black and white image. The cells were identified by using the 'analyze particles' feature with 'outlines' chosen in the settings which will open a new window showing a summary of the count of particles (cells), along with the total area and the average size number. To measure the percentage of fluorescent cells, the following **Equation 4** was used:

$$\%\ Fluorescence = \left(\frac{mCherry\ positive\ cells}{Hoechst\ stained\ cell}\right) \times 100$$

## SUPPLEMENTARY TABLES

### Table S1 – CRISPR-Cas9 sgRNA target sequences

| Custom LentiCRISPRv2 plasmids | Custom sequence | PAM | Source |
|---|---|---|---|
| LCV2_eGFP_12 | Oligo1:CACCGGGGCGAGGAGCTGTTCACCG<br>Oligo2: AAACCGGTGAACAGCTCCTCGCCCC | GGG | Shalem et al., 2014 |
| LCV2_ESR1_76 | Oligo7: CACCGCGCCGTGTACAACTACCCCG<br>Oligo8: AAACCGGGGTAGTTGTACACGGCGC | AGG | This study |

*Nucleotides in gray are Esp3I sticky ends, nucleotides in black are the sgRNA

### Table S2 – RNAi shRNA target sequences

| shRNA plasmid | Sequence (5'-3') | Source |
|---|---|---|
| Non-target shRNA | 5'- GCGCGATAGCGCTAATAATTT -3' | Dr. Sylvie Mader's lab |
| ERα shRNA 1 | 5'- CTACAGGCCAAATTCAGATAA -3' | Sigma-Aldrich TRCN0000003300 |

**Table S3 – Primers used in this study**

| Primer | Purpose | Sequence (5'–3') |
|---|---|---|
| LCV2_eGFP_12_F | Colony PCR | TTTTCTGCTCGCCGCTCAGGAA |
| LCV2_ESR1_76_F | Colony PCR | CACCGCGCCGTGTACAACTA |
| LCV2_cPCR_R | Colony PCR | TGTCCACCACTTCCTCGAAGTTCC |
| NeoRoverhang_F | Overhang PCR | TTGCATTCTAGACTGAGGCGGAAAGAACCAG |
| NeoRoverhang_R | Overhang PCR | CACAGCTTCTAGATGACGCTCAGTGGAACGA |
| LCMN_EcoRI_F | PCR amplification | TTGAATTCTAGACTGAGGCGGAAAG |
| LCMN_overhang_R | PCR amplification & overhang PCR | ACTGAACGTCTCTTAACGCGTCACTTGTACAGC |
| LCV2_overhang_F | PCR amplification & overhang PCR | TAGTTAAGAGACGCGTTAAGTCGACAATCAACCTCTG |
| LCV2_EcoRI_R | PCR amplification | TTCAAGACCTAGCTAGCGAATTCA |
| LCMNv2_cPCR_R | cPCR | CGCCAAAGTGGATCTCTGCTGTC |
| hU6_prom_F | Sequencing | GAGGGCCTATTTCCCATGATTC |
| NeoR_F | Sequencing | GAACAAGATGGATTGCACGC |

**Table S4 – 6-well plate template for lentiviral transduction for flow cytometry assessment of lentiviral titer**

| | | |
|---|---|---|
| Lentiviruses: 100 µL<br>Media: 400 µL | Lentiviruses: 50 µL<br>Media: 450 µL | Lentiviruses: 25 µL<br>Media: 475 µL |
| Lentiviruses: 12.5 µL<br>Media: 487.5 µL | Lentiviruses: 6.25 µL<br>Media: 493.75 µL | Lentiviruses: None<br>Media: 500 µL |

**Table S5 – Primers used for qRT-PCR**

| Gene-specific primers | Sequence (5'-3') |
|---|---|
| ERα - Forward | TTGACCCTCCATGATCAGGTC |
| ERα - Reverse | GCAAACAGTAGCTTCCCTGG |
| β-actin - (Forward & reverse) | Proprietary (ThermoFisher) |

**Table S6 – Primers used for gene cleavage detection assays**

| Gene target amplification – primer | Sequence (5'-3') | Product size (bp) | Expected cleaved bands' size (bp) |
|---|---|---|---|
| eGFP - Forward | GCCTCTGCCTCTGAGCTATTC | 433 | 293 & 160 |
| eGFP - Reverse | TGAAGAAGATGGTGCGCTCC | | |
| ERα - Forward | CATGACCCTCCACACCAAAG | 409 | 235 & 174 |
| ERα - Reverse | TTCTCCAGGTAGTAGGGCAC | | |

## SUPPLEMENTARY FIGURES

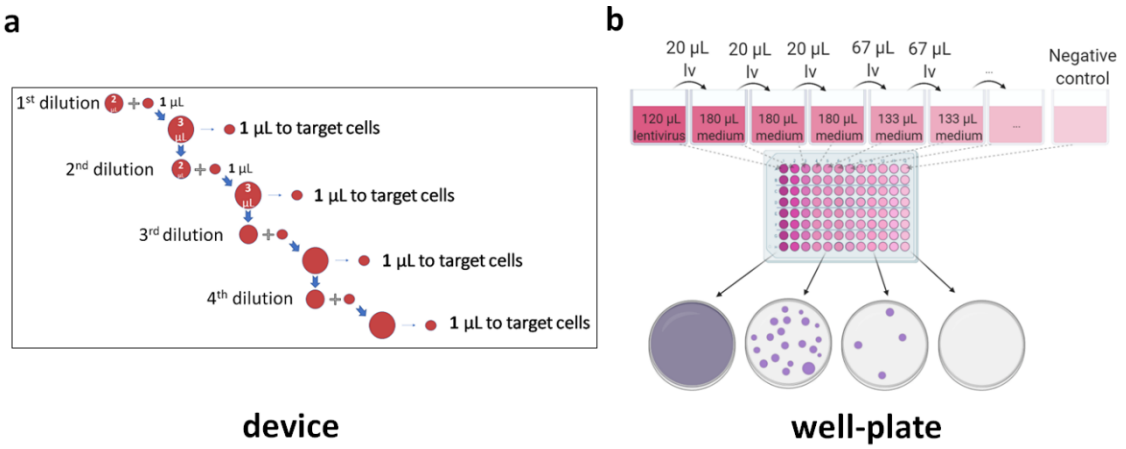


**Figure S1 - Lentiviral transduction dilutions to measure mean viral titer in the device and well-plate.** (a) Serial dilutions of 1:3, 1:6, 1:12, and 1:24 of lentiviral supernatant are formed at the production area following a dilution protocol. Each 1 µL unit DMEM droplet is applied to HEK293T cells in a serial fashion. A dilution of 1:3 (DMEM and viral titers) was implemented by merging 1 µL of DMEM with 2 µL of the supernatant containing the viruses. 1 µL of the merged product was split and actuated to a 'target cell' region while the remainder (2 µL) was saved for other dilutions. This procedure was repeated three times to generate dilutions 1:6, 1:12, and 1:24. After monitoring for two days, viral titer via the fluorescence of target cells was calculated based on Equation 1. (b) Serial dilutions of lentiviruses (lv) are applied to HEK293T cells in a 96-well plate. Colonies were allowed to grow in the presence of the puromycin for 7-8 days with medium renewal each 2 days and stained with 0.1 % w/v crystal violet in 20% ethanol and counted when the negative control well was completely dead. Counted colonies were converted into transducing units per mL via Equation 2 shown in the methodology.

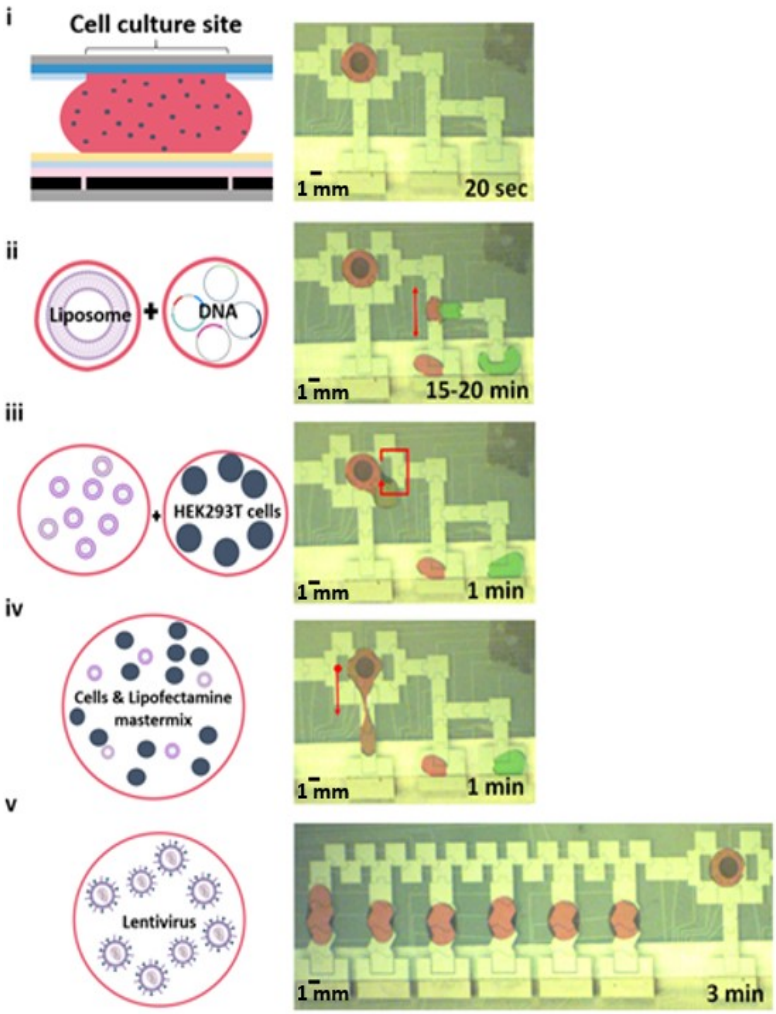


**Figure S2 - Lentiviral production and transduction strategy on the LENGEN platform.** The schematic shows the events at the genetic level with an image-based representation of the droplet movements (containing food dye) on the device. Left: the graphical representation of the five-step lentiviral generation, production, transduction, and analysis (drawings are not to scale). Right: images from a movie for each of the five steps along with the time taken for each step. Coloured food dyes were used to show the droplet. Step (i) HEK293T cells in a droplet are seeded at the production region. (ii) Plasmids and lipids from separate reservoirs are dispensed and merged and incubated for 20 mins. (iii) The DNA and lipids are dispensed into the HEK293T cell culture region and are mixed in a circular fashion around the adjacent electrodes. (iv) Excess mixture (containing plasmid, lipids, media) is dispensed away from the HEK293T cell culture region for removal. (v) After seeding the target cell line at the cell culture regions, unit droplets containing lentiviral particles with the plasmid of interest is split from the production region and actuated to a cell culture region (transduction). Analysis of the cells can be performed via microscopy after 24 or 48 h. For transduction analysis, cells can be removed by trypsin and cultured in well-plates for verification analysis (e.g., qRT-PCR, gene cleavage) or for sorting and isoclonal expansion (not shown).

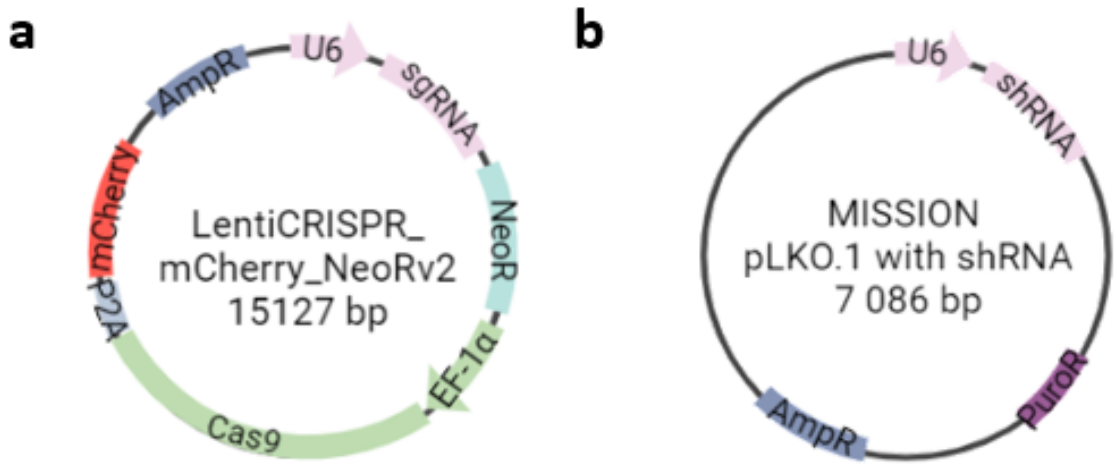


**Figure S3 - Transfer plasmids used in this study.** (a) LentiCRISPR_mCherry_NeoRv2 (LCMNv2) is a viral transfer vector that contains an sgRNA (targeting either eGFP or ESR1) under the promoter U6 and a spCas9 gene cassette linked to a fluorescent reporter, mCherry, via a P2A linker which are all under the promoter EF-1α. A neomycin/kanamycin resistance was assembled into the plasmid. (b) MISSION® pLKO.1 constructs contain an shRNA targeting ESR1 under the promoter of U6 and has a puromycin resistance cassette. The first plasmid is deposited into the online Addgene repository (Cambridge, MA).

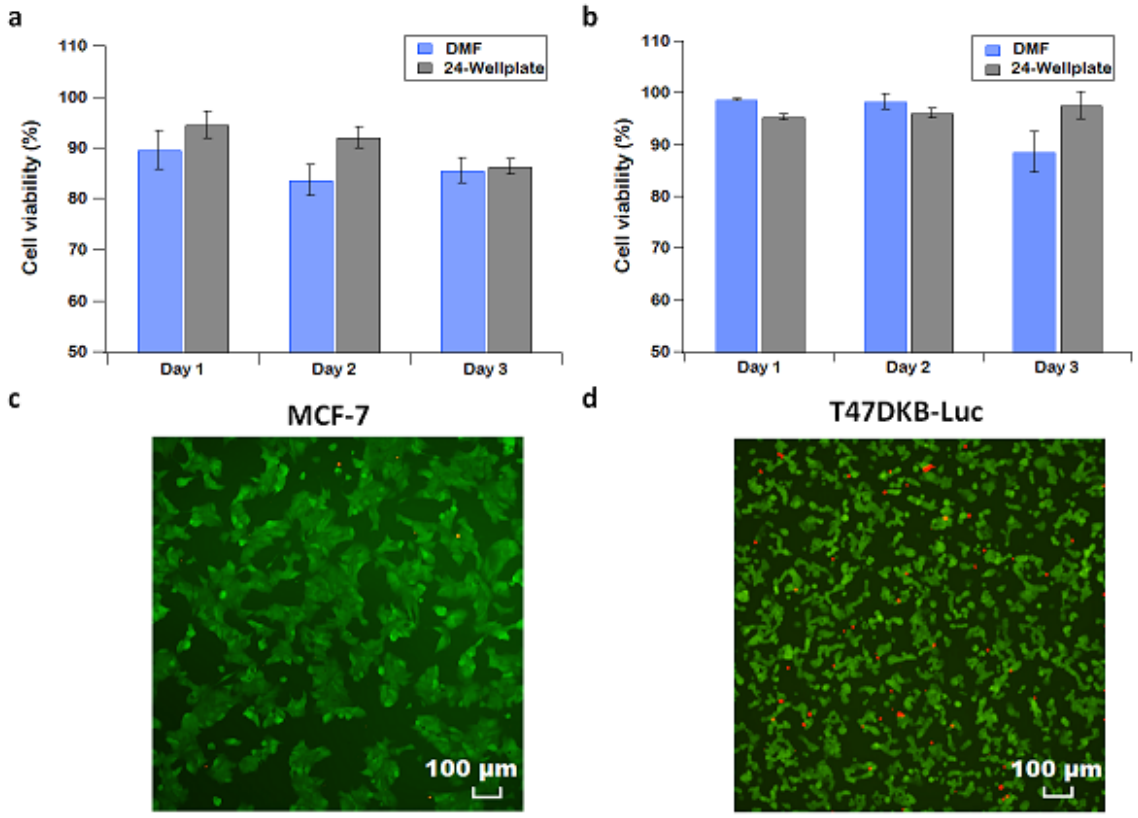


**Figure S4 - Cell viability of breast cancer cells' (MCF-7 & T47DKB-Luc) on DMF and 24-wellplate throughout 3 days.** (a-b) Viability bar graph of MCF-7 and T47DKB-Luc cells. An average viability of 83.7 and 85.7 % for MCF-7 and 98.4 and 88.6 % for T47DKB-Luc was observed on the device respectively. (c-d) Qualitative images of cell viability for breast cancer cells stained with FDA/PI in 24-well plate after 3 days.

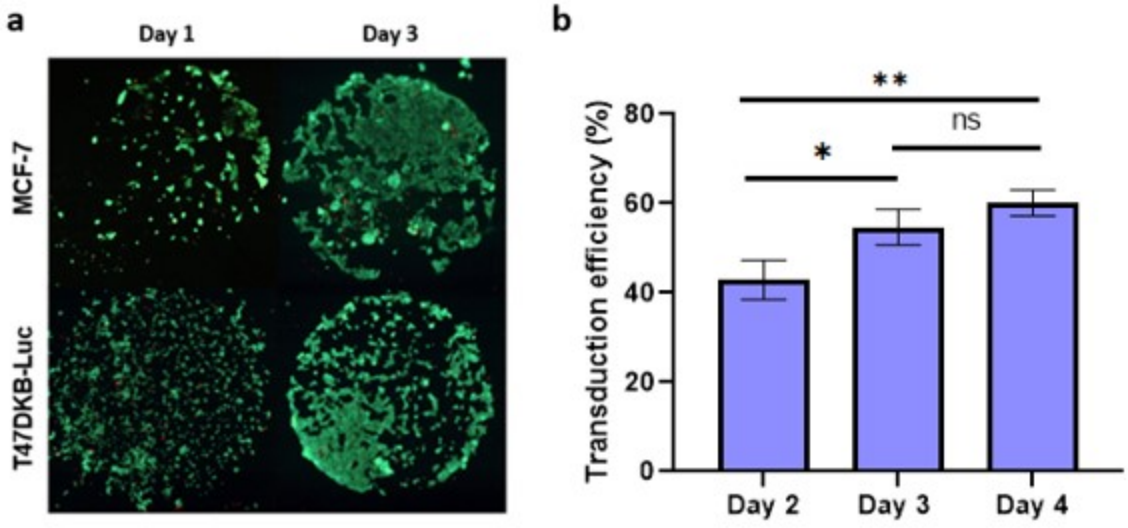


**Figure S5 - Optimizing viability and transduction efficiency for breast-cancer cell lines (MCF-7 and T47DKB-Luc) on the LENGEN device.** (a) Fluorescent images of each cell line treated with fluorescein diacetate (green) stains live cells and propidium iodine (red) stains for dead cells on the cell culturing sites for day 1 and 3. (b) Graph showing the transduction efficiency on T47DKB-Luc with a pLV-mCherry plasmid on the LENGEN platform for days 2-4. A student's t-test ($P < 0.05$) was used to evaluate the significance (* for a $P \leq 0.05$, ** for a $P \leq 0.01$ and ns for $P > 0.05$) between each day. Error bars represent ± 1 S.D. with N = triplicates.

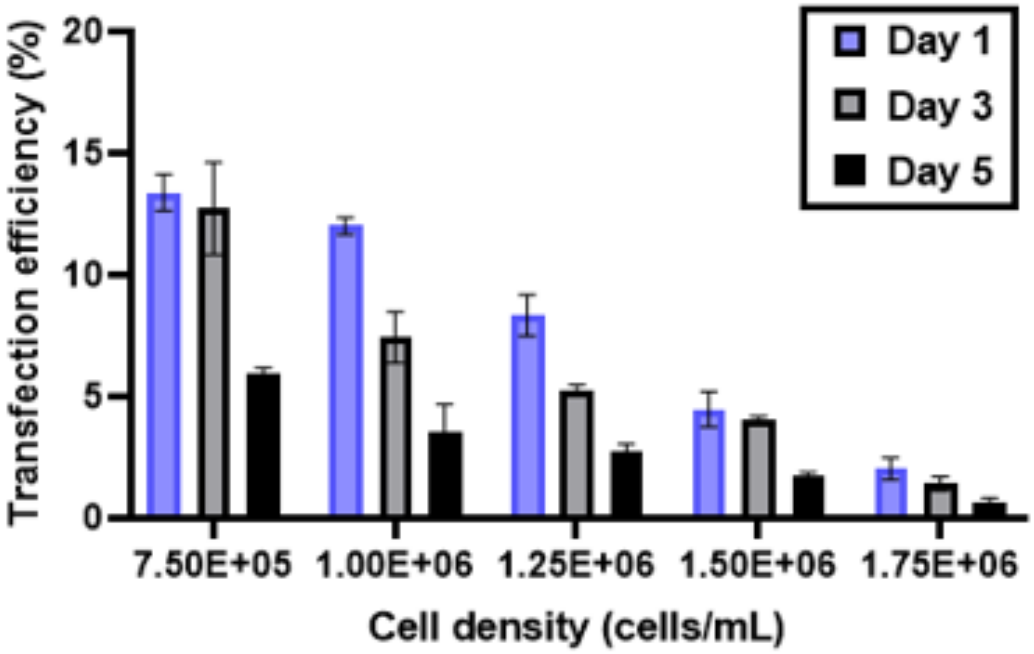


**Figure S6 - Lipid-mediated transfection efficiency of T47DKB-Luc with plasmid eGFP-N1 in 24-wellplate over 5 days.** Different cell densities were seeded in a well-plate for evaluation of transfection efficiency based on the observation of eGFP fluorescence in T47DKB-Luc cells. 750 ng of DNA plasmid was transfected into the cells. Within the first day, the highest transfection efficiency was observed at the lowest cell density is ~ 13.4%. Error bars for both plots represent ± 1 S.D. with N = triplicates.

**Sample calculations:**

For dilution 1:3 on microscale, seeding density is 3 x $10^6$ cells/mL with an average viral titer of 3.68 x $10^6$ TU/mL after **24 hours**

$$\frac{3.68 \times 10^6\ TU/mL}{3 \times 10^6\ cells/ml} \approx 1.23\ TU\ per\ cell$$

For dilution 1:3 on microscale, seeding density is 3 x $10^6$ cells/mL with an average viral titer of 1.05 x $10^7$ TU/mL after **48 hours**

$$\frac{1.05 \times 10^7\ TU/mL}{3 \times 10^6\ cells/ml} \approx 3.55\ TU\ per\ cell$$

For dilution 3:10 on macroscale, seeding density is 5 x $10^6$ cells/mL with an average viral titer of 1.4 x$10^7$ TU/mL after **5 days** of harvesting

$$\frac{1.4 \times 10^7 TU/mL}{5 \times 10^6 cells/ml} \approx 2.81\ TU\ per\ cell$$

**Figure S7 – Sample calculations for measuring the transducing units packaged per producer cell.** The cell seeding density was divided by the obtained mean viral titer to calculate the approximate transducing units packaged per producer cell on macroscale and microscale. The mean viral titer is obtained through plots in Figure 2b-c.

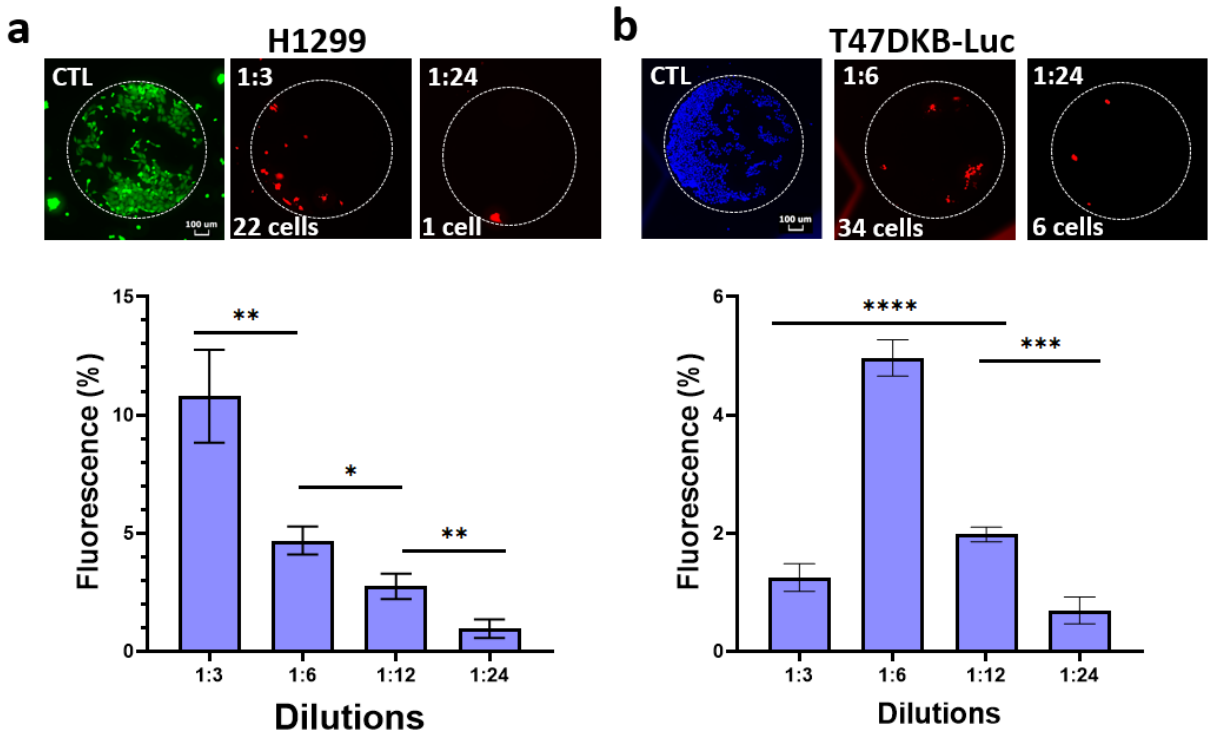


**Figure S8 - Evaluating the effect of a large lentiviral payload (~15 kb) for different cell lines: (a) H1299 and (b) T47DKb-Luc.** Each plot is accompanied with pictures showing the cells seeded on the hydrophilic spot (white outline) situated on the LENGEN device. Wild-type H1299 cells contained eGFP integrated into the cells (from Genecopeia) and wild-type T47DKb-Luc were fluorescently labeled with Hoechst 33342 stain. All cells were transduced with the lentiviral particles (at different dilutions 1:3, 1:6, 1:12, and 1:24) containing an mCherry fluorescent reporter. (+)mCherry cells were counted to generate the % fluorescence efficiency. A student's t-test ($P < 0.05$) was used to evaluate the significance (shown as * for a $P \leq 0.05$, ** for a $P \leq 0.01$, *** for a $P \leq 0.001$, **** for a $P \leq 0.0001$) between the dilutions. Error bars for both plots represent ± 1 S.D. with N = triplicates.

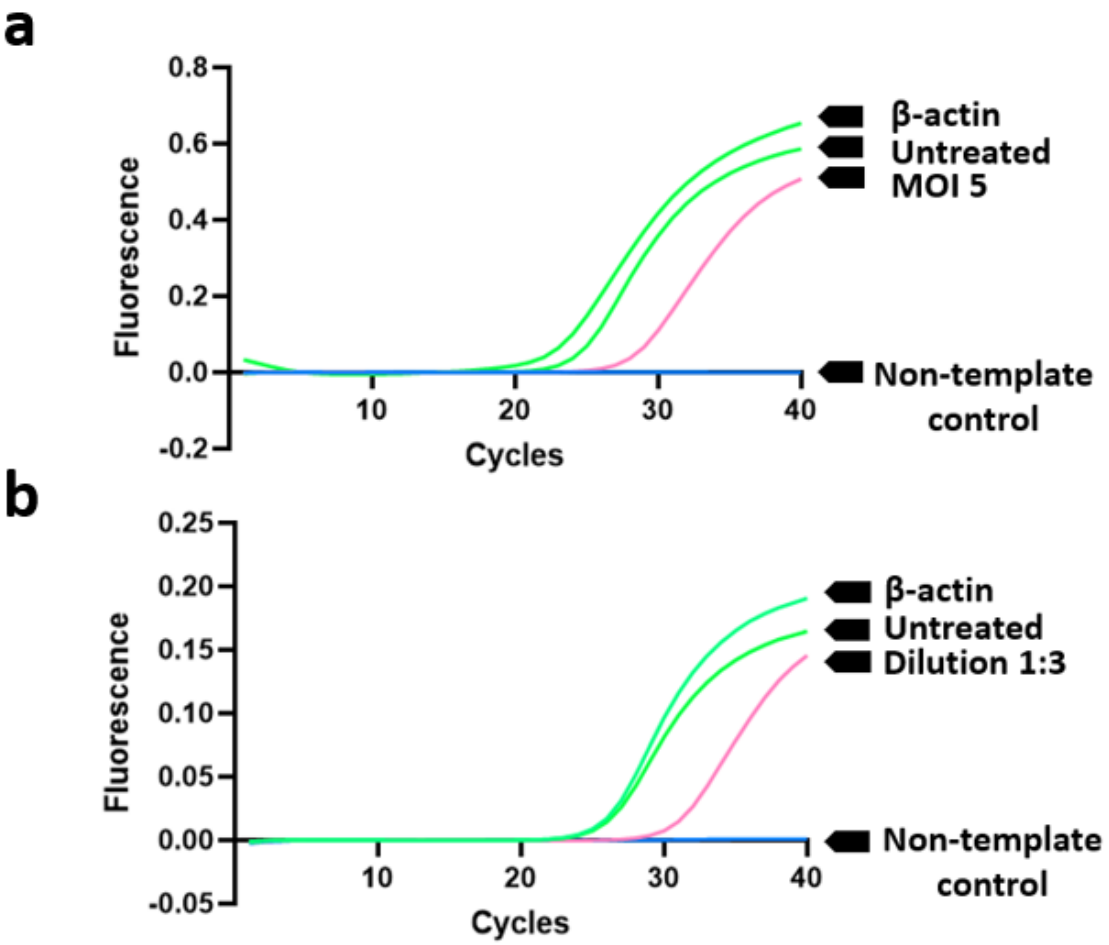


**Figure S9 - RT-qPCR raw data examples: RNAi assay on MCF-7's on LENGEN and in a 96-well plate.** Amplification plots measuring gene expression of ESR1 and ACTB (as a reference gene) in MCF-7 cells from (a) LENGEN and (b) well-plate platforms. Pooled 1:3 dilutions were transferred into a well-plate for analysis. All cells grown in well-plates were selected using puromycin antibiotic after seven days.

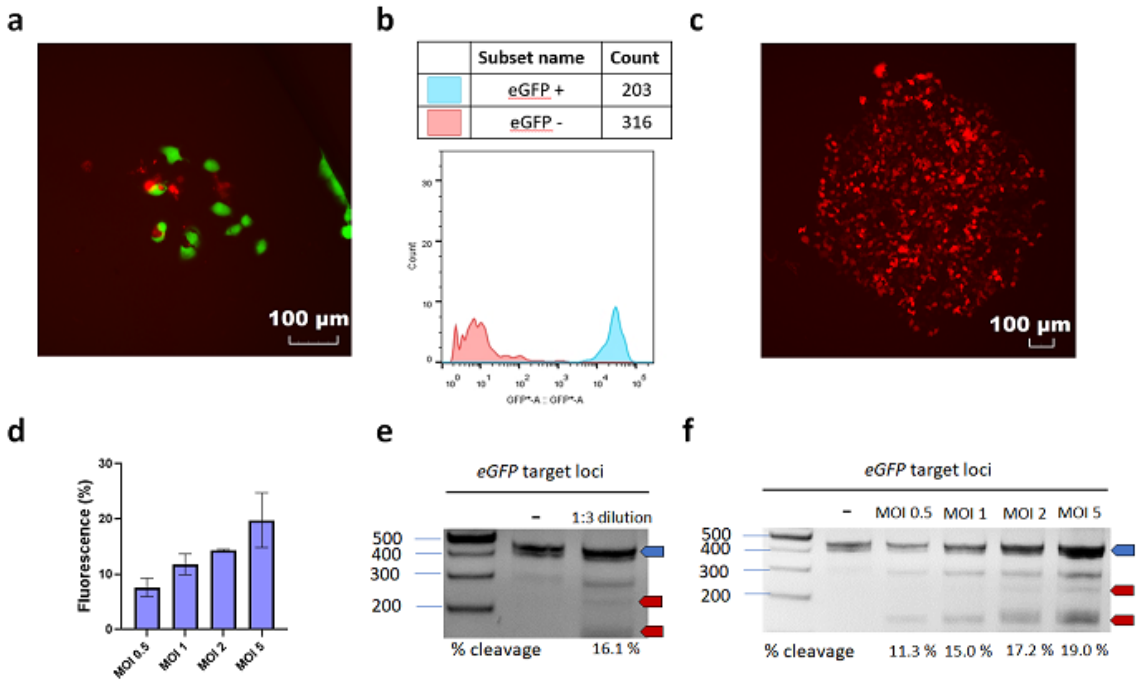


**Figure S10 - Expansion of eGFP knockout cells transduced on LENGEN on device and in well-plate.** (a) Pooled dilutions (1:3) of H1299 transduced on device transferred into a 96-well plate. Overlay images of mCherry and eGFP images show the knockout of eGFP in some cells five days post-transduction. (b) Cell sorting of H1299 grown from pooled dilutions (1:3). Flow cytometry data showing the count of eGFP knockout cells versus (+)eGFP cells. (c) Single cell expansion of (-)eGFP/(+)mCherry H1299 cells after ~2 weeks post-sorting. The heterogenous population was cell sorted for single cell expansion of knocked out eGFP and integrated mCherry-expressing cells. (d) Plot of fluorescence percentage at different MOIs (0.5, 1, 2 and 5) was measured in H1299 cells, two days post-transduction in a 96-well plate. Error bars represent ± 1 S.D. with N = triplicates. Genomic cleavage assay after seven days post-transduction on H1299 cells transduced with (e) the 1:3 dilution generated on LENGEN and (f) the indicated MOIs in well-plates along with a negative control (non-targeting sgRNA). The wildtype band is 433 bp (shown by the blue arrow arrow) and the expected cleavage bands are 293 bp and 160 bp (shown by the red arrows). There is presence of un-specific bands (~400 bp and ~310 bp) due to many repeated sequences located near the target loci.

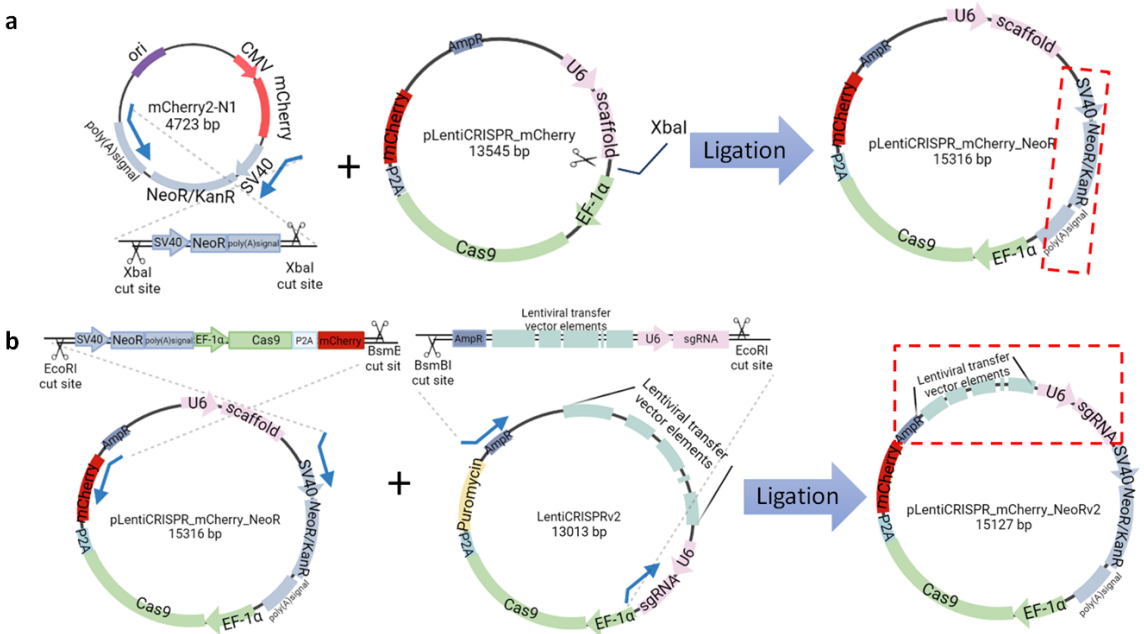


**Figure S11 – Schematic showing the two-step cloning procedures for the construction of pLentiCRISPR_mCherry_NeoRv2.** Cloning steps shown for (a) the construction of pLentiCRISPR_mCherry_NeoR by adding gene fragments of the neomycin resistance to the plasmid pLentiCRISPR_mCherry and (b) the construction of pLentiCRISPR_ mCherry_NeoRv2 by ligating part of the pLentiCRISPR_mCherry_NeoR (neomycin resistance, Cas9-P2A-mCherry) to part of the LentiCRISPRv2 plasmid (ampicillin resistance, lentiviral transfer vector elements and the sgRNA). The dotted box shows the inserted part into the backbone.

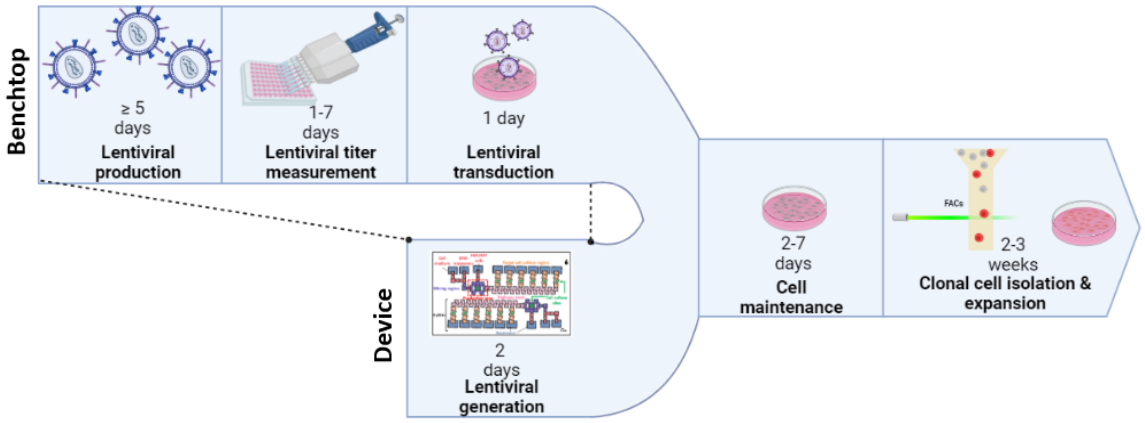


**Figure S12 – Schematic of the lentiviral generation process on benchtop versus device.** The timescale of the lentiviral generation process done on benchtop and on device are compared. The generation process includes production, titration measurement (e.g., selection/plaques or FACS or qPCR[6-7]) and transduction. Both are concluded with the cell maintenance, clonal cell isolation and expansion steps.

## References


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