ADVANCED SCIENCE
Open Access

Supporting Information

for *Adv. Sci.*, DOI 10.1002/advs.202505474

Genetic Manipulation of Mammalian Cells in Microphysiological Hydrogels

*Anna C. Jäkel, Dong-Jiunn Jeffery Truong and Friedrich C. Simmel**

Genetic Manipulation of Mammalian Cells in Microphysiological Hydrogels

Supporting Information

Anna C. Jäkel,[†] Dong-Jiunn Jeffery Truong,[‡,¶] and Friedrich C. Simmel[*,†]

[†]*TU Munich, School of Natural Sciences, Department of Bioscience, Garching, Germany*

[‡]*Institute for Synthetic Biomedicine, Helmholtz Munich, Neuherberg, Germany*

[¶]*Department of Bioscience, TUM School of Natural Sciences, Technical University of Munich, Munich, Germany*

E-mail: simmel@tum.de

Phone: +49 89 289 11610. Fax: +49 89 289 11612

Doxycycline Titration

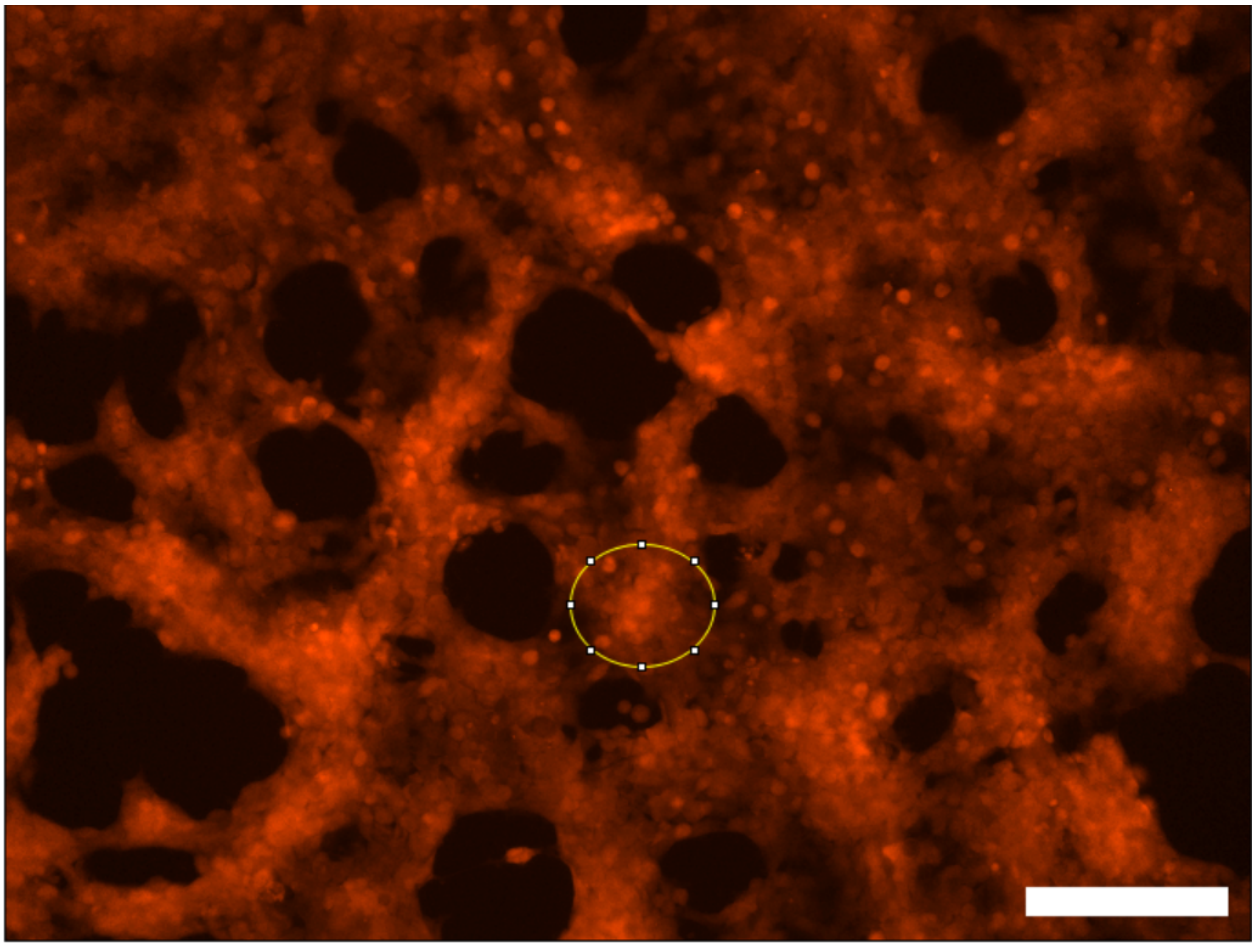
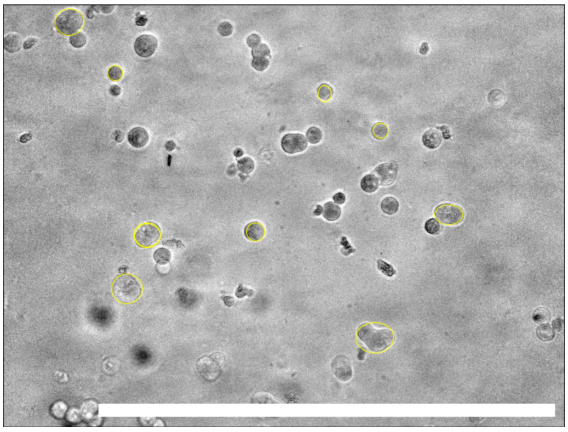
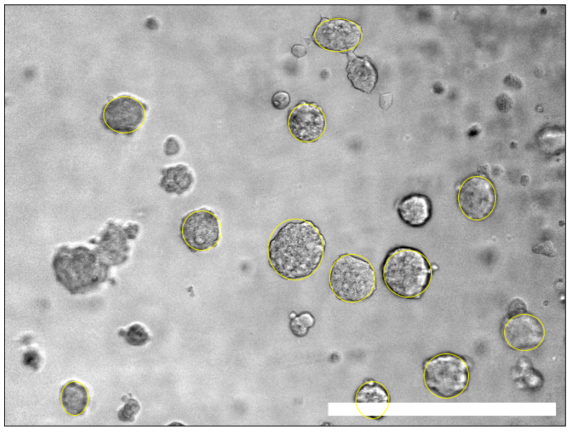


Figure S1: A region of interest (ROI) was selected for each sample that was $100\,\%$ confluent. The following parameters were measured for over a period of $60\,\mathrm{h}$: area, mean intensity, standard deviation, min and max intensity. Measurements were taken every $2\,\mathrm{h}$ for the first $24\,\mathrm{h}$, then after $24\,\mathrm{h}$, and then after $12\,\mathrm{h}$. Scalebar: $100\,\mu\mathrm{m}$

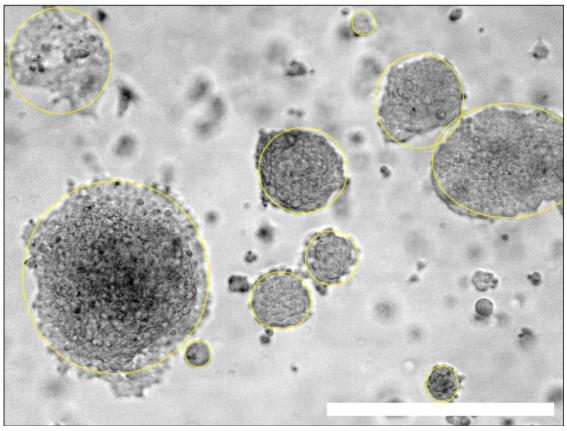
Cell growth in GelMa



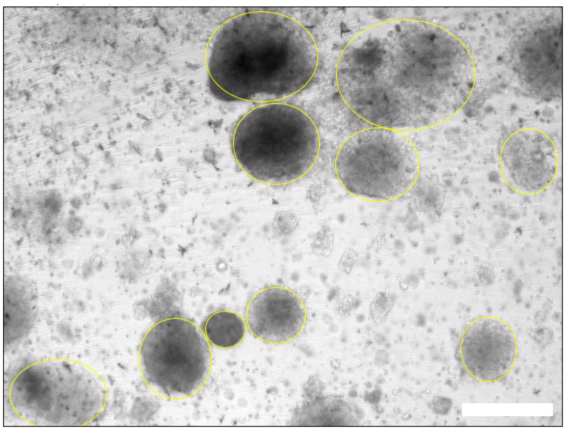
(a) Day 1 after seeding cells in GelMa.



(b) Day 5 after seeding cells in GelMa.

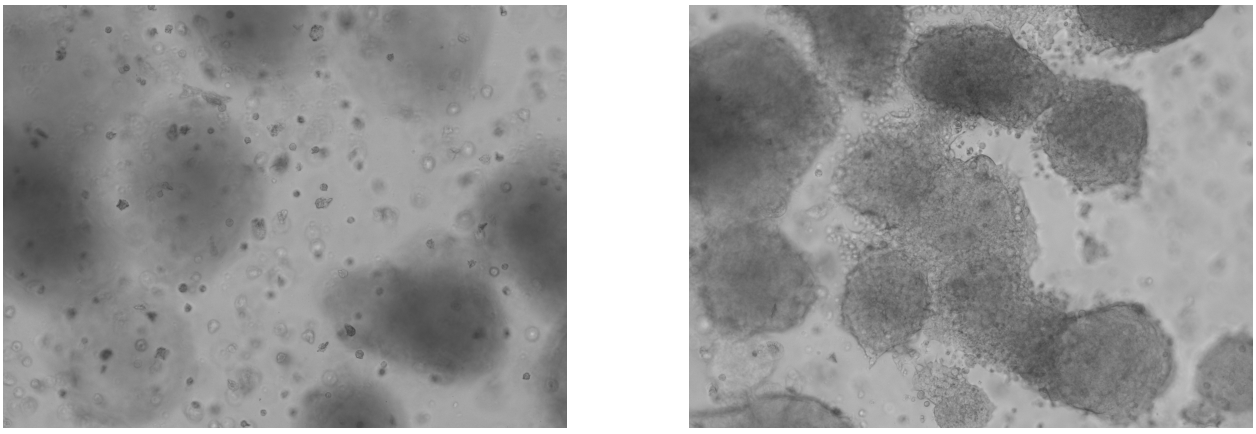


(c) Day 8 after seeding cells in GelMa.



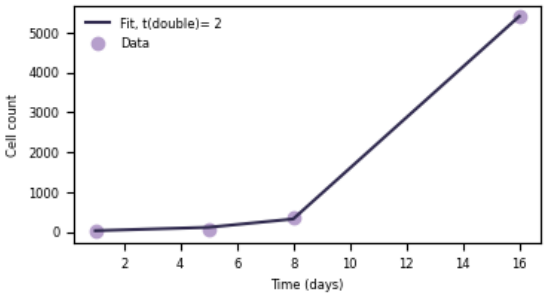
(d) Day 16 after seeding cells in GelMa.

Figure S2: For each sample 10 clusters were selected to measure cluster size. The feret radius was determined with Fiji and the mean was calculated for each sample. Scalebar: $500\,\mu m$

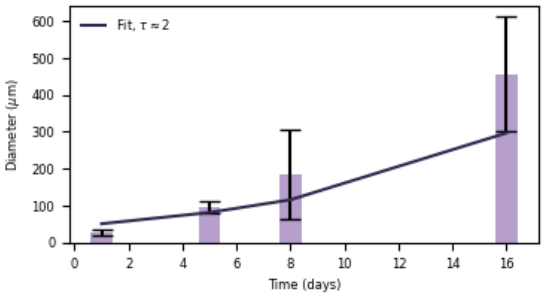


(a) (b)

Figure S3: Cells were cultured for 8 days in $\approx 500\,\mu m$ thick GelMA. Shown are two images focusing on a lower and higher layer within the gel. a) The cells in lower layers (more than $200\,\mu m$ away from the surface) were not sufficiently supplied with DMEM and did not grow further. b) The cells in the upper layers, down to $\approx 200\,\mu m$ depths, did grow into large cell clusters.



(a) Number of cells calculated in one cell cluster over time.



(b) Cell cluster diameter over time. Fitting the data results in a doubling time of ≈ 2 days.

Figure S4: The cell count N_c was calculated assuming a spherical volume of each cluster. And the doubling time was calculated using the formula $N_c(t) = N_0 \times 2^{t/t_D}$.

Transfection

Transfection of mCherry - FACS

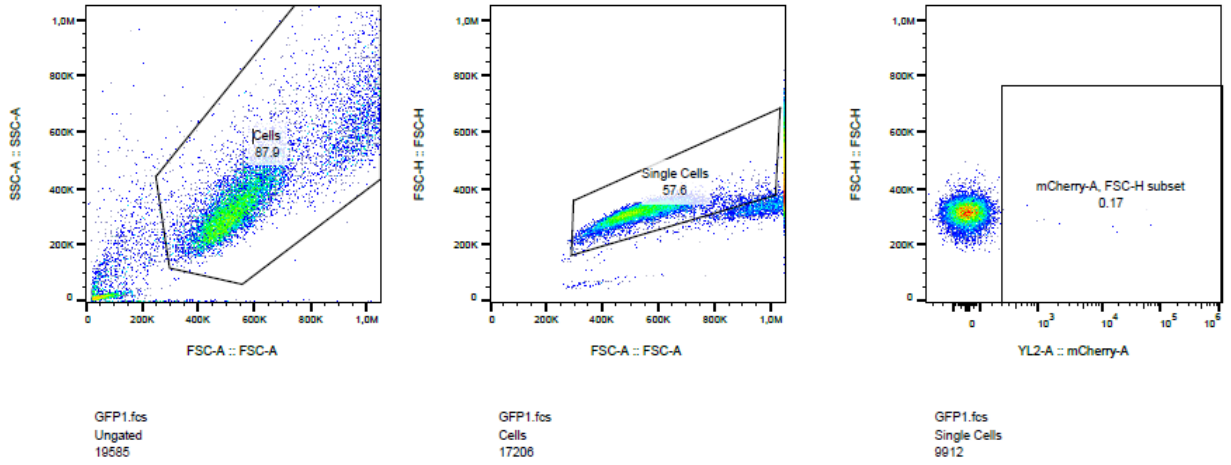


Figure S5: Control measurement of HEK293T cells.

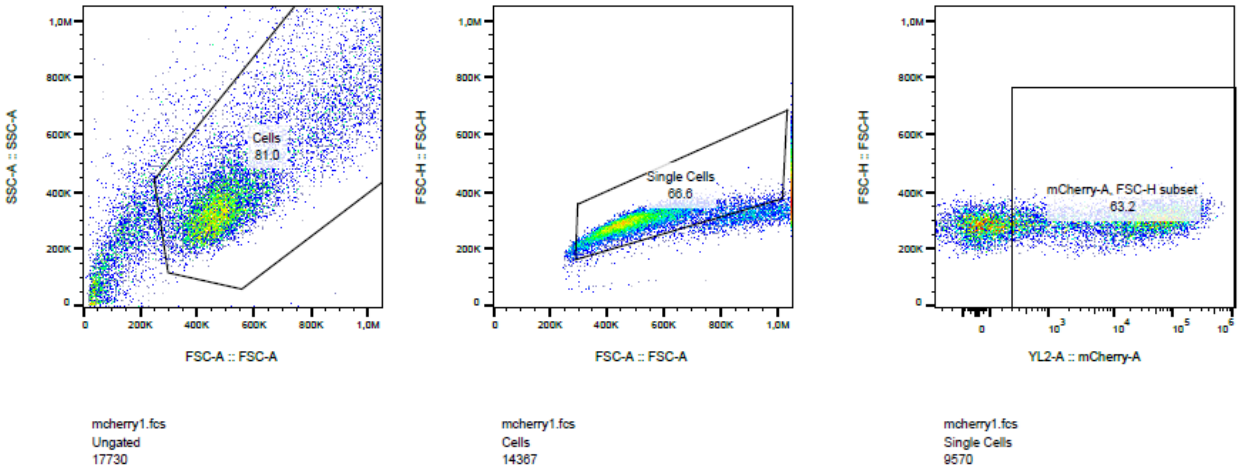


Figure S6: HEK293T were transfected using jetOptimus transfection reagent with plasmid DNA encoding for the fluorescent protein mCherry.

Rheometer Measurements

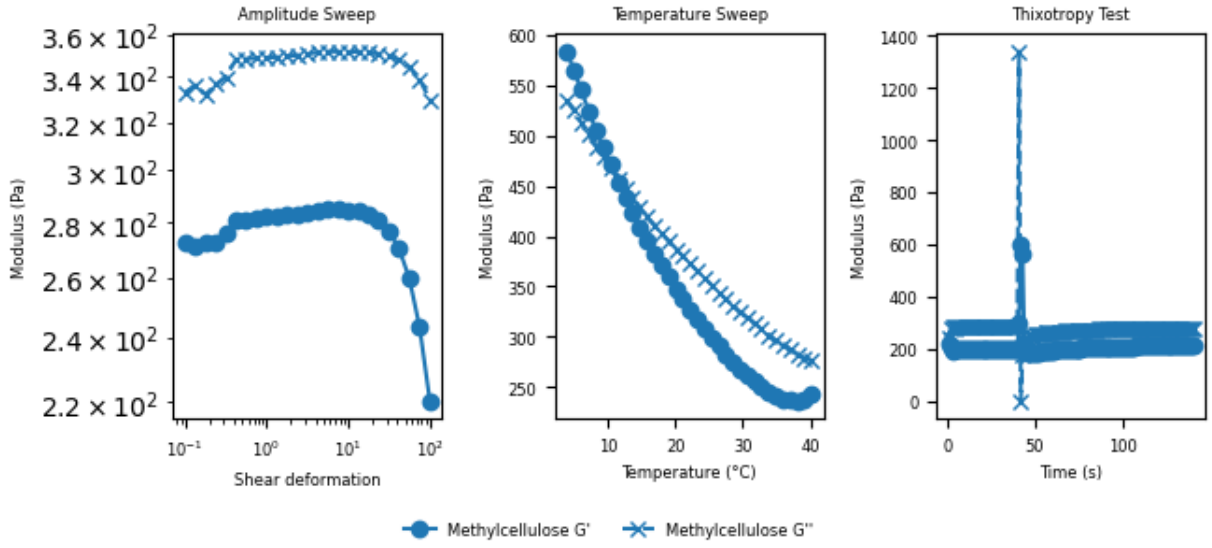


Figure S7: Methylcellulose ($5\,\%$ v/w) was measured as benchmark. The amplitude sweep provides insight into the viscoelastic properties of methylcellulose by evaluating its storage modulus (G') and loss modulus (G'') across increasing strain amplitudes. In the linear viscoelastic region (LVR), G'' remains higher than G', indicating that the material can be described as a viscoelastic liquid. As strain increases beyond the critical strain, G' decreases, and the limit of the LVE-region is reached. This suggests that methylcellulose maintains its gel-like properties under low deformation but undergoes yielding at higher strains. The temperature-dependent rheological behavior of methylcellulose is characterized by a decrease in G' at higher temperatures, indicative of thermogelling properties. Initially, at lower temperatures, the material behaves more elastic with $G' > G''$. As temperature rises, the viscous proportion predominates, reflected in a crossover point where G' surpasses G''. This transition temperature is a critical parameter for applications requiring thermal responsiveness. The thixotropy test assesses the recovery of methylcellulose after shear-induced breakdown. Upon applying high shear, both G' and viscosity drop significantly, reflecting structural disruption. When shear is reduced, a gradual recovery of G' is observed, indicating partial structural reformation. However, if full recovery is not achieved within the measured timeframe, this suggests that methylcellulose exhibits a degree of irreversible structural breakdown or slow rebuilding dynamics. This behavior is relevant for applications where shear-induced fluidization and recovery kinetics are important.

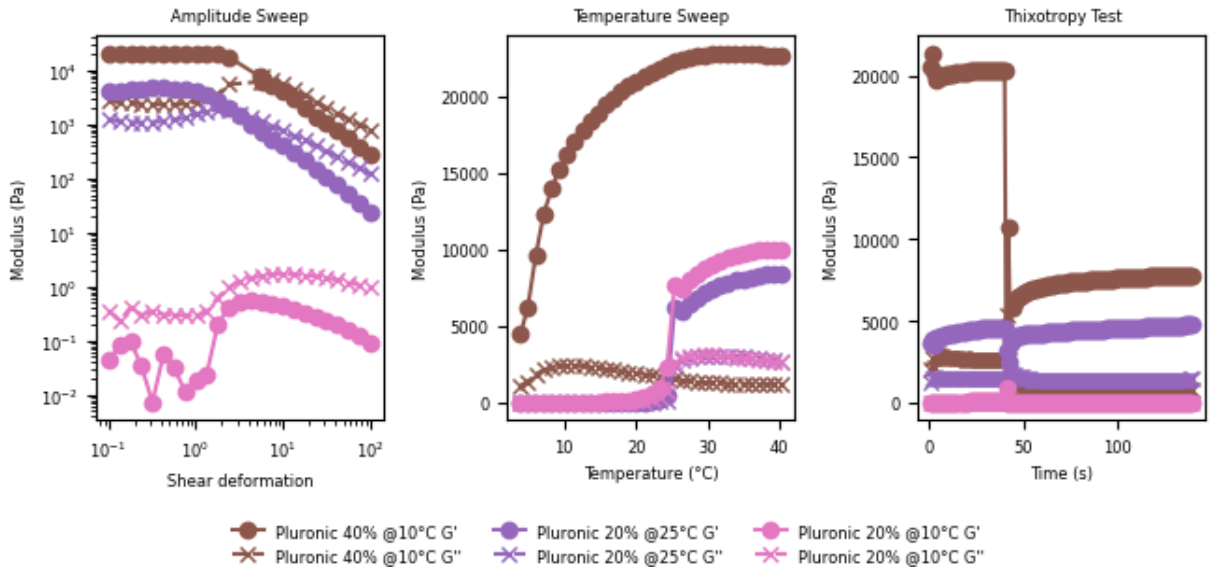


Figure S8: Pluronic is printable at $20\,\%$ and $40\,\%$ at room temperature for $\sim 15\,$min when stored before at $-4\,^{\circ}$C. An amplitude sweep of pluronic hydrogels at different concentrations and temperatures are shown. Compared to methylcellulose (Figure S7), the pluronic curves exhibit a less steep decline in the storage modulus (G') and loss modulus (G'') with increasing strain, indicating a more gradual structural breakdown. Additionally, pluronic $20\,\%$ at $10\,^{\circ}$C shows significantly lower G' values compared to pluronic $20\,\%$ at room temperature or pluronic $40\,\%$, suggesting a temperature-dependent shift in mechanical strength. This behavior reflects the thermoresponsive nature of pluronic, where gel stiffness varies with both concentration and temperature. Interestingly pluronic with a concentration of $40\,\%$ at $10\,^{\circ}$C behaves similar as pluronic with a concentration of $20\,\%$ at $25\,^{\circ}$C. The temperature sweep of pluronic hydrogels at different concentrations shows that in contrast to methylcellulose, which shows a more linear increase in modulus with temperature, pluronic exhibits a non-monotonic "hill-shaped" curve. The storage modulus (G') initially increases with rising temperature, reaches a peak, and then declines at higher temperatures. This trend suggests a gelation process followed by structural weakening, likely due to micellar rearrangement or phase separation. Additionally, the difference between storage (G') and loss modulus (G'') is more pronounced compared to methylcellulose, highlighting distinct viscoelastic behavior and phase transitions in pluronic hydrogels. The thixotropy test shows that plurnic at $40\,\%$ does not recover its structure and viscosity as the $20\,\%$ pluronic does.

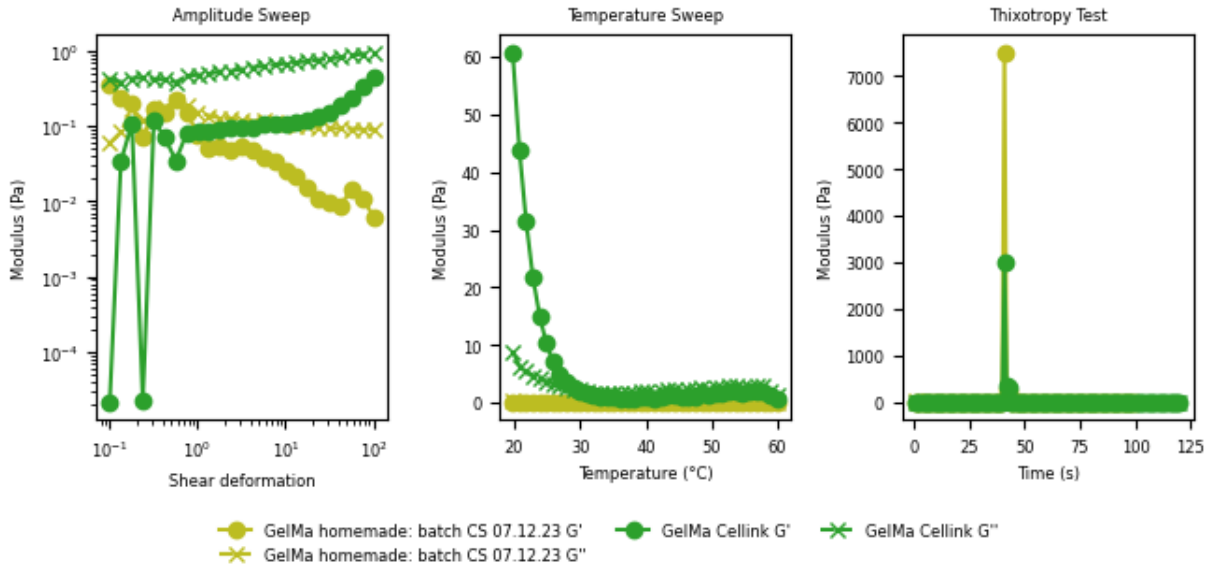


Figure S9: Rheological analysis of GelMa purchased from Cellink and our homemade GelMa. Amplitude-, temperature sweep, and thixotropy test. The temperature sweep shows similar behaviour of the hydrogels above $30\,^{\circ}\mathrm{C}$. The thixotropy test shows that both hydrogels recover its structure and viscosity after deformation.

Cell viability in different gels

Cell line test

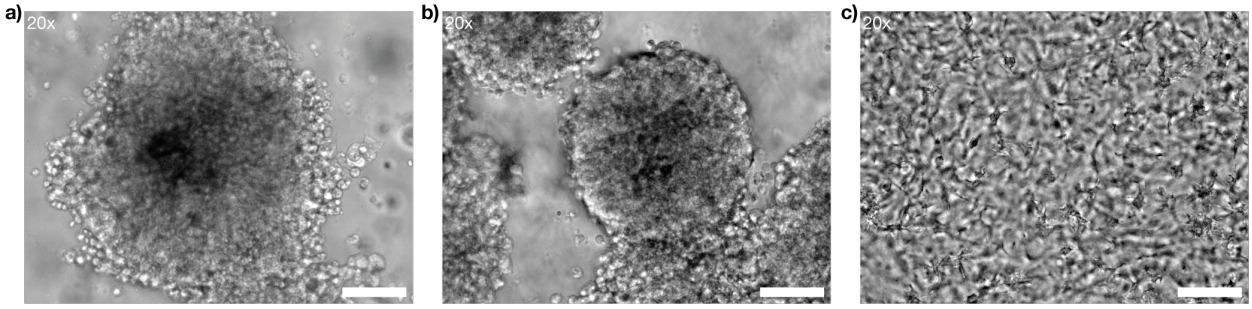


Figure S10: Different celltypes grown in GelMa for 16 days: a) HEK293T (ATCC, CRL-3216™) b) NIH-3T3 (ATCC, CRL-1658™) c) hMSC (The cells were a kind gift from the Clausen-Schaumann lab). Scalebar: $100\,\mu m$.

Compatibility with glue

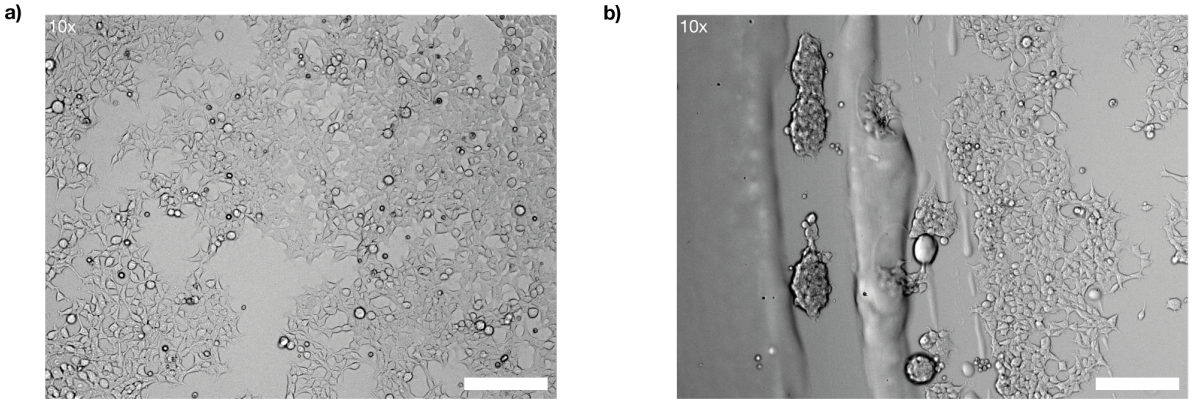


Figure S11: a) Cell growth in a well plate next to a glue drop. b) Cell growth inside the gel drop. Cells do grow in clusters similar to cells growing in GelMa. Scalebar: $200\,\mu m$.

Test of nozzle precision

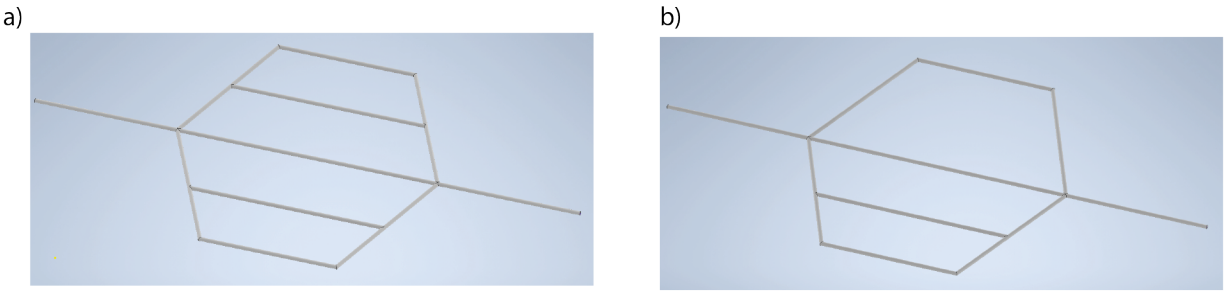


Figure S12: Printed vascular structures. a) Even distribution network. b) Uneven distribution network.

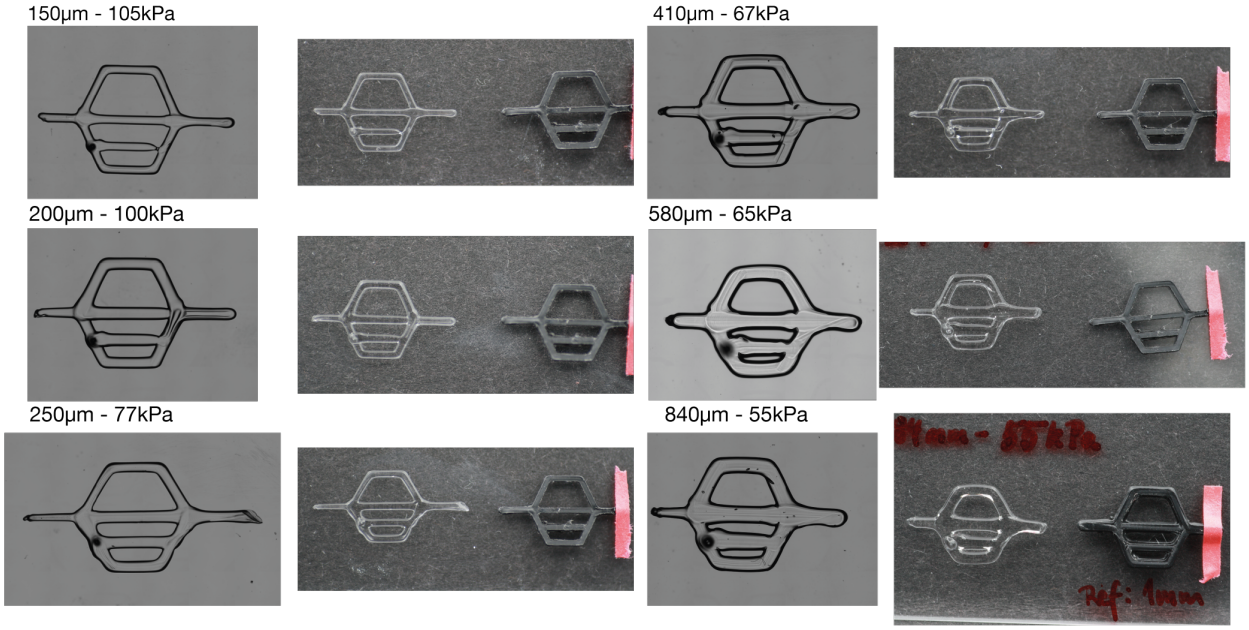


Figure S13: Channel structures printed with a BIOX2 printer (Cellink). Different needle sizes were tested and images are presented of the structure for each needle with the optimal pressure value. Right: microscope image, left: photograph of the structure next to a PLA printed construct which was printed with a Bambulab X1E printer. The channel thickness was 1 mm to compare resolution.

Fluorescein Channel Experiments

To fabricate vasculature-like structures, a $40\,\%$ (w/v) Pluronic F-127 solution was prepared by dissolving pluronic powder (Sigma-Aldrich) in double-distilled water (ddH_2O) at $4\,^{\circ}$C overnight, with periodic vortexing to ensure complete dissolution. This temperature-responsive bioink served as a sacrificial support material, facilitating the creation of vascular channels within the GelMa matrix.

A BIOX 3D bioprinter (Cellink) equipped with a 200 µm nozzle was used to print the vascular structures. The pluronic solution, stored at $4\,^{\circ}$C, was printed at room temperature, maintaining its optimal viscosity (cf. Figure S8). To prevent premature gelation, printing was performed within 15 minutes of removing the solution from cold storage. If the solution warmed beyond a critical threshold, re-cooling was necessary before continuing the process. Printing parameters were optimized, including a pressure of $100\,$kPa, a printing speed of $5\,\mathrm{mm\,s^{-1}}$, and a preflow adjustment of $-50\,$ms to ensure controlled extrusion.

The bioprinting workflow for generating vascular-like channels is illustrated in Figure S14. First, Pluronic F-127 ($40\,\%$ w/v) was printed as a sacrificial ink to define the channel structure within GelMa. Figure S14a presents a photograph of a pluronic-printed channel stained in red, to highlight the channel structures within the hydrogel. The complete bioprinting process (Figure S14b) involved printing pluronic structures, overlaying the construct with GelMa, and crosslinking the hydrogel using $405\,$nm UV light for $30\,$seconds. Following crosslinking, the constructs were cooled to $4\,^{\circ}$C for 5 minutes, liquefying the pluronic and enabling its removal, thereby creating perfusable channels.

To validate the functionality of these channels, Figure S14c shows a brightfield image of a pluronic structure before GelMa casting, demonstrating the precision and integrity of the printed channels. Additionally, Figure S14d presents fluorescence imaging of fluorescein diffusion from the channels into the hydrogel, confirming molecular transport into the surrounding matrix. This approach establishes a controlled environment for studying molecular diffusion and cellular responses within engineered hydrogel constructs.

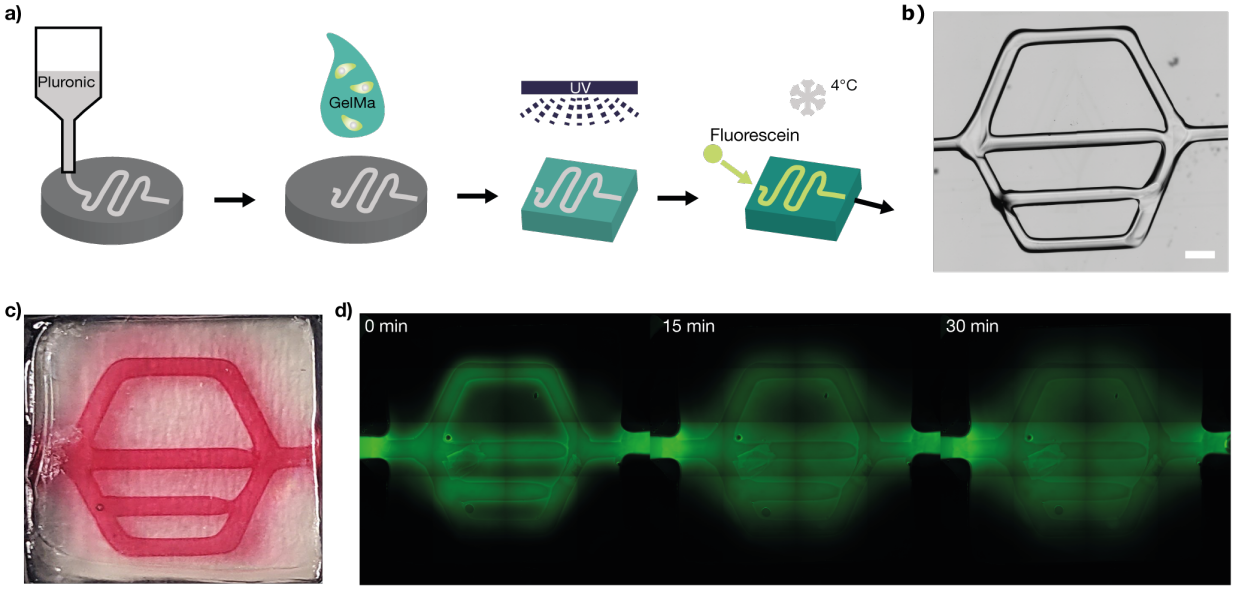


Figure S14: Construction of vascular-like structures. a) Schematic representation of the method for preparing vascular structures using a commercial 3D bioprinter. Pluronic F-127 is printed, followed by casting GelMa with or without cells on top. The GelMa is crosslinked using $405\,\mathrm{nm}$ UV light for $30\,\mathrm{seconds}$. Subsequently, the construct is cooled to $4\,^{\circ}\mathrm{C}$ for 5 minutes, liquefying the pluronic to enable channel perfusion with a material of interest. b) Brightfield image of the printed channel before hydrogel application. Scale bar: $1000\,\mathrm{m}$. c) Photograph of a constructed channel filled with red food dye to visualize perfusion within the GelMa matrix. d) Channels perfused with fluorescein to observe diffusion into the surrounding hydrogel over time. e) Diffusion of fluorescein from a single channel. Scale bar: $1000\,\mathrm{m}$. f) Quantitative analysis of fluorescein diffusion.

Bioreactor Setup

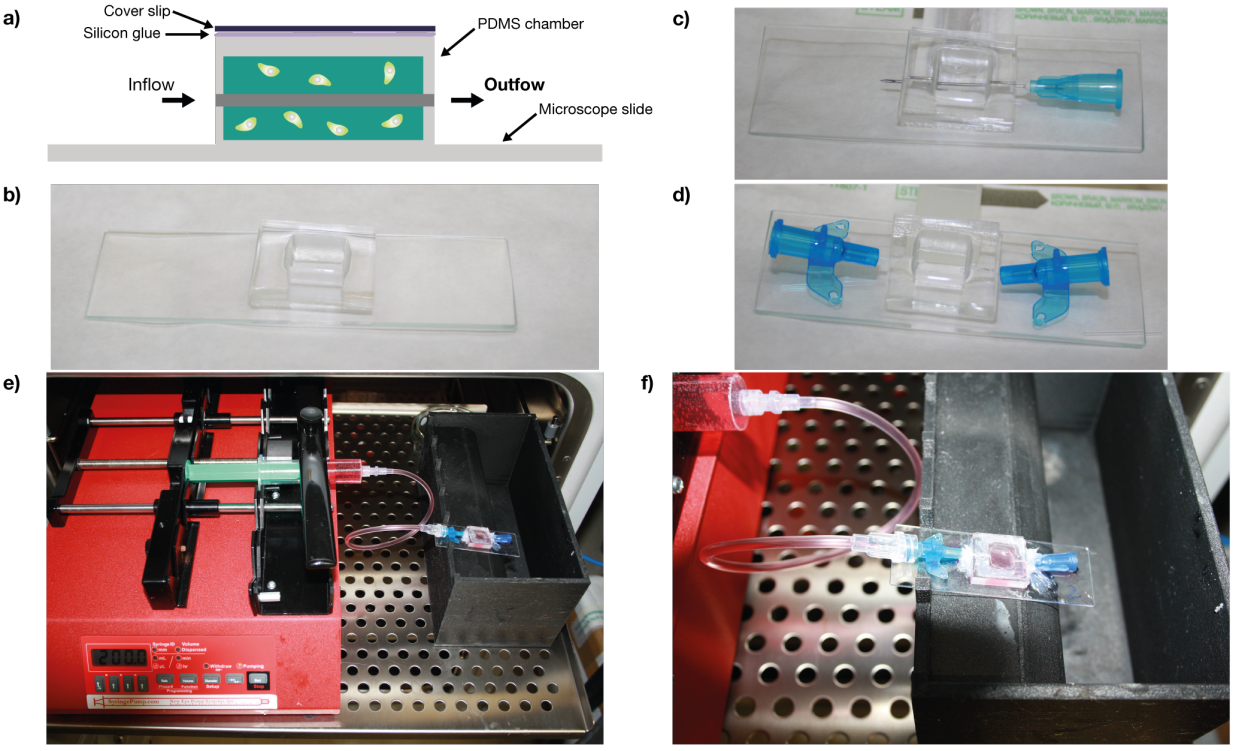


Figure S15: a) Schematic of the bioreactor. b) PDMA chamber bonded to a microscope slide with O2 plasma. c) A needle (ID $0.6\,\mathrm{mm}$) was punched through PDMS for casting gel on top for the creation of a channel. d) In- and outlet connection were achieved with catheters ($22\,\mathrm{G}$). e) Photograph of the whole setup. A syringe pump is used to be able to run 6 bioreactors in parallel. A 3D printed autoclavable PAHT chamber is used as collection tank for material flushed through the bioreactors. f) Zoom in on the bioreator itself.

Plasmids

The full sequence of the plasmid used for transfection studies of mCherry is provided below

cgcgatgtacgggccagatatacgcgttgacattgattattgactagttattaatagtaatcaattacggggtcattagttcatagccca
tatatggagttccgcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgac
gtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagtacatca
agtgtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatggg
actttcctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatagc
ggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaat
gtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctctctggctaactag
agaacccactgcttactggcttatcTTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGCgaaattaatacg
actcactatagggagacccaagctggctagcgtttaaacttaagcttggtaccgagctcggatccactagtccagtgtggtggaattcg
ccaccatggtgagcaagggcgaggaggataacatggccatcatcaaggagttcatgcgcttcaaggtgcacatggagggctccgtga
acggccacgagttcgagatcgagggcgagggcgagggccgcccctacgagggcacccagaccgccaagctgaaggtgaccaaggg
tggcccccctgcccttcgcctgggacatcctgtcccctcagttcatgtacggctccaaggcctacgtgaagcaccccgccgacatccccg
actacttgaagctgtccttccccgagggcttcaagtgggagcgcgtgatgaacttcgaggacggcggcgtggtgaccgtgacccagg
actcctccctgcaggacggcgagttcatctacaaggtgaagctgcgcggcaccaacttcccctccgacggccccgtaatgcagaagaa
gaccatgggctgggaggcctcctccgagcggatgtaccccgaggacggcgccctgaagggcgagatcaagcagaggctgaagctga
aggacggcggccactacgacgctgaggtcaagaccacctacaaggccaagaagcccgtgcagctgcccggcgcctacaacgtcaac
atcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtacgaacgcgccgagggccgccactccaccggcggc
atggacgagctgtacaagtgagcggccgctcgagtctagagggccgggtaaggagggcccgtttaaacccgctgatcagcctcgact
gtgccttctagttgccagccatctgttgtttgcccctcccccgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaata
aaatgaggaaattgcatcgcattgtctgagtaggtgtcattctattctgggggggtggggtggggcaggacagcaagggggaggattg
ggaagacaatagcaggcatgctggggatgcggtgggctctatggctcgctttcttgctgtccaatttctattaaaggttcctttgttccct
aagtccaactactaaactggggatgcggccgctcgagtctagagatccggtgtggaaagtccccaggctccccagcaggcagaagta
tgcaaagcatgcatctcaattagtcagcaaccaaagctctagagatccggtgtggaaagtccccaggctccccagcaggcagaagtat

gcaaagcatgcatctcaattagtcagcaaccaaagctttaaaccatccggtgtggaaagtccccaggctccccagcaggcagaagtat
gcaaagcatgcatctcaattagtcagcaaccaaagctttaaacccgctgatcagcctcgactacaacaaggcaaggcttgaccgacaa
ttgcatgaagaatctgcttagggttaggcgttttgcgctgctttgtgacattaagcgcggcgggtgtggtggttacgcgcagcgtgacc
gctacacttgccagcgccctagcgcccgctcctttcgctttcttcccttcctttctcgccacgttcgccggctttccccgtcaagctctaa
atcgggggctccctttagggttccgatttagtgctttacggcacctcgaccccaaaaaacttgattagggtgatggttcacgtagtgggc
catcgccctgatagacggtttttcgccctttgacgttggagtccacgttctttaatagtggactcttgttccaaactggaacaacactcaa
ccctatctcggtctattcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgatttaacaaaaatttaacg
cgaattttaacaaaatattaacgcttacaatttaggtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttctaaatac
attcaaatatgtatccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaaggaagagtatgagtattcaacatttcc
gtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaagatca
gttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagattttcgccccgaagaacgttttccaatgat
gagcacttttaaagttctgctatgtggcgcggtattatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactattctca
gaatgacttggttgagtactcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataacc
atgagtgataacactgcggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatc
atgtaactcgccttgatcgttgggaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggc
aacaacgttgcgcaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatggaggcggataaagttg
caggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctggagccggtgagcgtgggtctcgcggtatcattg
cagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtcaggcaactatggatgaacgaaatagac
agatcgctgagataggtgcctcactgattaagcattggtaactgtcagaccaagtttactcatatatactttagattgatttaaaacttca
tttttaatttaaaaggatctaggtgaagatccttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcag
accccgtagaaaagatcaaaggatcttcttgagatccttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccag
cggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtccttc
tagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggctgctg
ccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggataaggcgcagcggtcgggctgaacgggggttt
cgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccg
aagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcct
ggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcctatggaaaa

acgccagcaacgcggccttttttacggttcctggccttttgctggccttttgctcactcatt

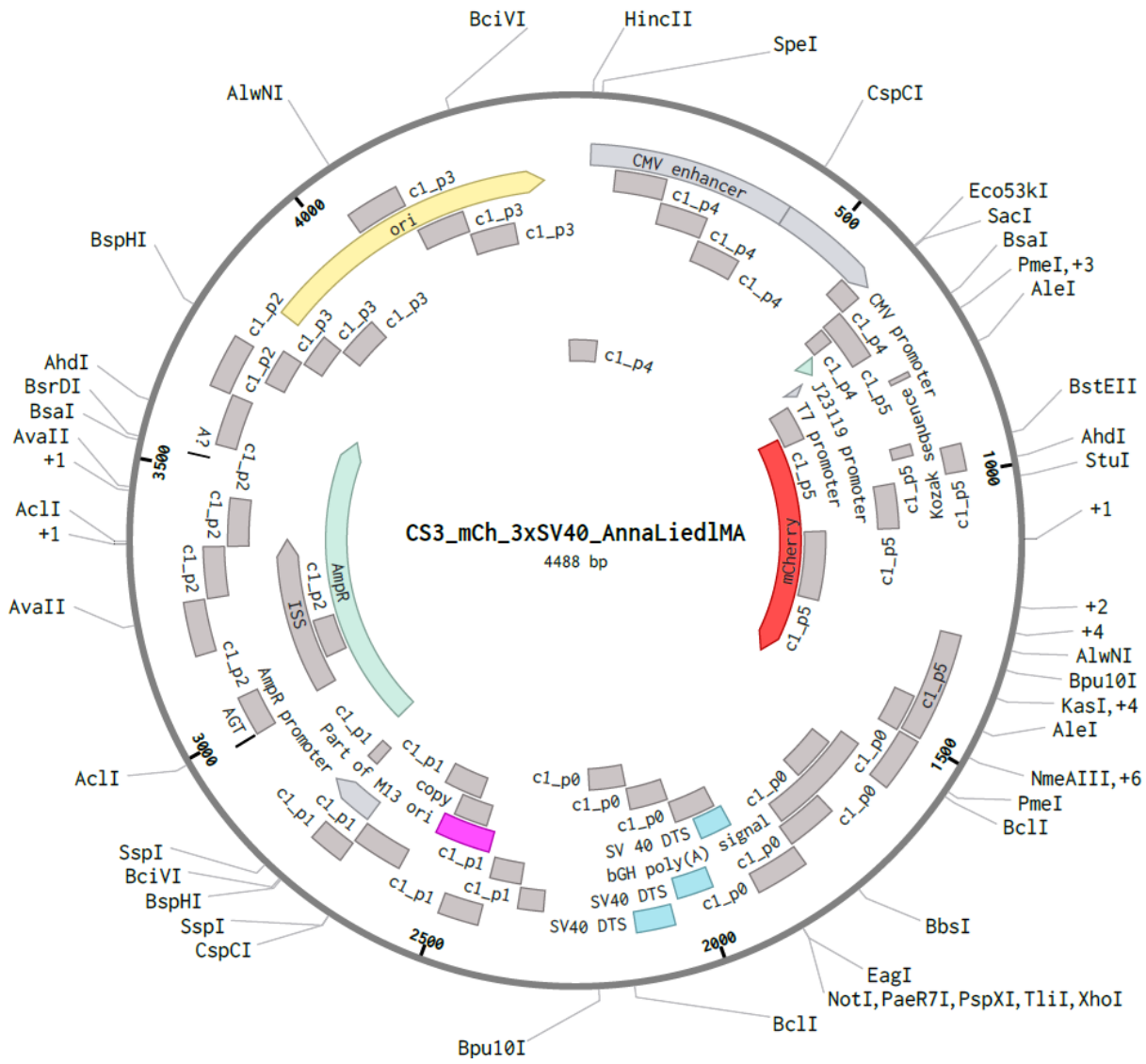


Figure S16: Plasmid used for transfection experiments. The fluorescent protein mCherry is expressed as reporter. The plasmid was a kind gift from the Dietz lab engineered by Anna Liedl, already published in this reference (*1*).

The full sequence of the plasmid that was used to create the stable cell line HEK293T that expresses mScarlet-I upon doxycycline induction is provided below

gtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataacc
ctgataaatgcttcaataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattccctttttgcggcattttgcc
ttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtgggttacatcgaactggat
ctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggcgcggta
ttatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcaccagtcacaga
aaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttacttctga
caacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccggagct
gaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaact
acttactctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctg
gctggtttattgctgataaatctggagccggtgagcgtggatctcgcggtatcattgcagcactggggccagatggtaagccctcccgt
atcgtagttatctacacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagc
attggtaactgtcagaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatcctt
tttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttga
gatccttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagagctacca
actcttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtagccgtagttaggccaccacttcaag
aactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttg
gactcaagacgatagttaccggataaggcgcagcggtcgggctgaacgggggttcgtgcacacagcccagcttggagcgaacgac
ctacaccgaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggtaa
gcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacc
tctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggccttttacggttcctg
gcctttgctggccttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgatacc
gctcgccgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcccaatacgcaaaccgcctctccccg
cgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagtta

gctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacagg

aaacagctatgaccatgattacgccaagcgcgtgtatacttaaccctagaaagatagtctgcgtaaaattgacgcatgcattcttgaaa

tattgctctctctttctaaatagcgcgaatccgtcgctgtgcatttaggacatctcagtcgccgcttggagctcccgtgaggcgtgcttgt

caatgcggtaagtgtcactgattttgaactataacgaccgcgtgagtcaaaatgacgcatgattatcttttacgtgacttttaagatttaa

ctcatacgataattatattgttatttcatgttctacttacgtgataacttattatatatatattttcttgttatagatatcgtgactaatatat

aataaaggccggccgccctgcaggtttaaatgatttgccctcccatatgtccttccgagtgagagacacaaaaaattccaacacactat

tgcaatgaaaatacatttcctttattagccagaagtcagatgcaggcccaaggttttgctttttttttttttaagaaaggccaaaaagcaa

aacctgagactttcgcctcaggaaaagaaaaaccttccggcaagaagcatggccaccgaggctccagcgtcgactcacccggggagc

atgtcaaggtcaaaatcgtcaagagcgtcagcaggcagcatatcaaggtcaaagtcgtcaagggcatcggctgggagcatgtctaag

tcaaaatcgtcaagggcgtcggtcggcccgccgctttcgcactttagctgtttctccaggccacatatgattagttccaggccgaaaag

gaaggcaggttcggctccctgccggtcgaacagctcaattgcttgtttcagaagtgggggcatagaatcggtggtaggtgtctctcttt

cctcttttgctacttgatgctcctgttcctccaatacgcagcccagtgtaaagtggcccacggcggacagagcgtacagtgcgttctcca

gggagaagccttgctgacacaggaacgcgagctgattttccagggtttcgtactgtttctctgttgggcgggtgccgagatgcacttta

gccccgtcgcgatgtgagaggagagcacagcggtatgacttggcgttgttccgcagaaagtcttgccatgactcgccttccaggggggc

aggagtgggtatgatgcctgtccagcatctcgattggcagggcatcgagcagggcccgcttgttcttcacgtgccagtacagggtagg

ctgctcaactcccagcttttgagcgagtttccttgtcgtcaggccttcgataccgactccattgagtaattccagagcagagtttatgact

ttgctcttgtccagtctagacatggtggcggcccgggcacagctggggagagaggtcggtgattcggtcaacgagggagccgactgc

cgacgtgcgctccggaggcttgcagaatgcggaacaccgcgcgggcaggaacagggcccacactaccgccccacaccccgcctccc

gcaccgccccttcccggccgctgctctcggcacgccctgctgagcagccgctattggccacagcccatcgcggtcggcgcgctgccat

tgctccctggcgctgtccgtctgcgagggtactagtgagacgtgcggcttccgtttgtcacgtccggcacgccgcgaaccgcaaggaa

ccttcccgacttaggggcggagcaggaagcgtcgccgggggggcccacaagggtagcggcgaagatccgggtgacgctgcgaacgg

acgtgaagaatgtgcgagacccagggtcggcgccgctgcgtttcccggaaccacgcccagagcagccgcgtccctgcgcaaacccag

ggctgccttggaaaaggcgcaactccaaccccgtggcggccgcgatcgaattccgtctcacgcgccggattcgacattgattattgact

agttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcct

ggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtca

atgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacg

gtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgtattagtcatcgctattac

catggtcgaggtgagccccacgttctgcttcactctccccatctccccccccctccccacccccaattttgtatttatttatttttttaattattt

tgtgcagcgatgggggcgggggggggggggggggcgcgcgccaggcggggcggggcggggcgagggggcggggcggggcgaggc

ggagaggtgcggcggcagccaatcagagcggcgcgctccgaaagtttccttttatggcgaggcggcggcggcggcggccctataaa

aagcgaagcgcgcggcgggcgggagtcgctgcgcgctgccttcgccccgtgccccgctccgccgccgcctcgcgccgcccgccccgg

ctctgactgaccgcgttactcccacaggtgagcgggcgggacggcccttctcctccgggctgtaattagcgcttggtttaatgacggct

tgtttcttttctgtggctgcgtgaaagccttgaggggctccgggagggccctttgtgcgggggggagcggctcgggggggtgcgtgcgtg

tgtgtgtgcgtggggagcgccgcgtgcggctccgcgctgcccggcggctgtgagcgctgcgggcgcggcgcggggctttgtgcgctc

cgcagtgtgcgcgaggggagcgcggccgggggcggtgccccgcggtgcgggggggggctgcgaggggaacaaaggctgcgtgcgg

ggtgtgtgcgtgggggggtgagcaggggggtgtgggcgcgtcggtcgggctgcaacccccccctgcacccccctccccgagttgctgag

cacggcccggcttcgggtgcggggctccgtacggggcgtggcgcggggctcgccgtgccgggcgggggggtggcggcaggtggggg

tgccgggcggggcggggccgcctcgggccggggagggctcgggggagggggcgcggcggcccccggagcgccggcggctgtcgag

gcgcggcgagccgcagccattgccttttatggtaatcgtgcgagagggcgcagggacttcctttgtcccaaatctgtgcggagccgaa

atctgggaggcgccgccgcacccccctctagcgggcgcggggcgaagcggtgcggcgccggcaggaaggaaatgggcggggaggg

ccttcgtgcgtcgccgcgccgccgtcccccttctccctctccagcctcggggctgtccgcgggggggacggctgccttcgggggggacgg

ggcagggcggggttcggcttctggcgtgtgaccggcggctctagagcctctgctaaccatgttcatgccttcttcttttttcctacagatc

cttaattaataatacgactcactataggggccgccaccatggtgagcaagggcgaggagctgttcaccggggtggtgcccatcctggt

cgagctggacggcgacgtaaacggccacaagttcagcgtccgcggcgagggcgagggcgatgccaccaacggcaagctgaccctga

agttcatctgcaccaccggcaagctgcccgtgccctggcccaccctcgtgaccaccttaggctacggcgtggcctgcttcgcccgctac

cccgaccacatgaagcagcacgacttcttcaagtccgccatgcccgaaggctacgtccaggagcgcaccatctctttcaaggacgacg

gcacctacaagacccgcgccgaggtgaagttcgagggcgacaccctggtgaaccgcatcgtgctgaagggcatcgacttcaaggag

gacggcaacatcctggggcacaagctggagtacaacttcaacagccacaaggtctatatcacggccgacaagcagaagaacggcatc

aaggctaacttcaagacccgccacaacgttgaggacggcggcgtgcagctcgccgaccactaccagcagaacacccccatcggcgac

ggccccgtgctgctgcccgacaaccactacctgagccatcagtccaaactgagcaaagaccccaacgagaagcgcgatcacatggtc

ctgaaggagagggtgaccgccgccgggattacacatgacatggacgagctctacaaatgaacgcgtcaagcacgcagcaatgcagc

tcaaaacgcttagcctagccacacccccacgggaaacagcagtgattaacctttagcaatatacgaaagtttaactaagctatactaac

cccagggttggtcaatttcgtgccagccacaccgtggatgcgcacccccctctccctcccccccccctaacgttactggccgaagccgc

ttggaataaggccggtgtgcgtttgtctatatgttattttccaccatattgccgtcttttggcaatgtgagggcccggaaacctggccctg

tcttcttgacgagcattcctaggggtctttcccctctcgccaaaggaatgcaaggtctgttgaatgtcgtgaaggaagcagttcctctgg

aagcttcttgaagacaaacaacgtctgtagcgacccttgcaggcagcggaacccccacctggcgacaggtgcctctgcggccaaaa

gccacgtgtataagatacacctgcaaaggcggcacaaccccagtgccacgttgtgagttggatagttgtggaaagagtcaaatggctc

tcctcaagcgtattcaacaaggggctgaaggatgcccagaaggtaccccattgtatgggatctgatctggggcctcggtgcacatgctt

tacatgtgtttagtcgaggttaaaaaacgtctaggccccccgaaccacggggacgtggttttcctttgaaaaacacgatgataatatgg

ccaccaccatgaccgagtacaagcctacagtgcggctggctaccagggacgatgtgccaagagctgtgcggacactggccgctgcct

tcgccgattaccctgccacaagacacaccgtggaccccgaccggcacatcgagagagtgaccgagctgcaagaactgtttctgactag

agtgggcctggacatcggcaaagtgtgggtggccgatgatggcgccgctgtggctgtgtggacaacccctgagtctgtggaagcagg

cgctgtgttcgccgagatcggacctagaatggccgagctgagcggctccagactggctgcccagcagcagatggaaggcctgctggc

cccccacagaccaaaagagcctgcctggtttctggctaccgtgggcgtgtcacctgaccaccagggcaagggactgggatctgctgtg

gtgctgcctggggtggaagctgctgaaagggctggcgtgcccgccttcctggaaacaagcgccccccagaaacctgcccttctacgag

agactgggcttcaccgtgaccgccgacgtggaagtgcctgagggccctagaacctggtgcatgaccagaaagcctggcgccggttcc

ggagctacaaacttcagcctgctgaaacaggctggcgacgtggaagagaaccccggtcctgcttcttatccttgtcaccaacacgcca

gcgccttcgatcaagccgctagaagcagaggccacagcaacagaagaacagccctcaggccaagaaggcagcaagaggctacaga

agtgcggctggaacagaagatgcccacactgctgagagtgtatatcgacggccctcatggcatgggcaagaccacaacaacacagct

gctggtggctctgggcagcagagatgacatcgtgtacgtgcccgagcctatgacctactggcaggtcctgggagcctctgagacaatc

gccaacatctacaccacacagcacagactggaccagggcgaaattagcgcaggcgacgctgctgtggtcatgacatctgcccagatc

acaatgggcatgccttacgccgtgacagacgctgtgctggctccacatattggcggcgaggctggatcttctcacgctccaccacctgc

tctgaccctgatcttcgacagacaccccatcttcgccctgctgtgttaccctgccgctcggtatctgatgggcagcatgacacctcaggc

cgtgctggctttcgtggctctgattcctcctacactgcccggcacaaacatcgtgcttggagccctgccagaggacagacacatcgaca

gactggctaagagacagaggcctggcgagagactggatctggctatgctggccgccatcagaagagtgtacggcctgctggctaaca

ccgtgcgctatcttcaaggcggcggttcctggagagaggactggggacagctctctggcacagcagttcctccacaaggcgctgagcc

tcagtctaacgctggacccagacctcacatcggcgacaccctgttcacactgttccgcgcccctgaactgctggcccctaacggcgacc

tgtataacgtgttcgcctgggctctcgacgtgctggcaaaaagactgcggcccatgcatgtgttcatcctggactacgatcagagccca

gccggatgcagagatgccctgctgcaactgacaagcggcatggtgcagacccatgtgacaacccctggcagcatccccaccatctgtg

acctcgccagaaccttcgctagagagatgggcgaagccaactaagtttaaacgctcgctttcttgctgtccaatttctattaaaggttcc

tttgttccctaagtccaactactaaactgggggatattatgaagggccttgagcatctggattctgcctaatataaaacatttatttcatt

gcgacgatcgtcagacatgataagatacattgatgagtttggacaaaccacaactagaatgcagtgaaaaaaatgctttatttgtgaaa
tttgtgatgctattgctttatttgtaaccattataagctgcaataaacaagttaacaacaacaattgcattcattttatgtttcaggttcag
ggggaggtgtgggaggttttttaaagcaagtaaaacctctacaaatgtggtaggcgcgtctacttgtacagctcgtccattccgcctgt
gctgtgtctcccctcgcttctctcgtactgttccaccacggtgtagtcctcgttgtggctggtgatgtccagctttctgtccacgttgtagg
cgccaggcatctgcacaggtttcttggccttgtaggtggtcttgaagtcggccaggtatctgccgccatccttcagtctcagggccatct
tgatgtcgcccttcagcacgccatcttcagggtacagtctctcggtgctggcttcccagcccattgtctttttctgcatcacagggccgtc
tggagggaagtttgtgccccgcagcttcactttgtagatcagggtcccatcttccagagatgtgtcctgtgtcacagtcacggctccgcc
gtcctcgaagttcatcactctctcccacttgaagccctctgggaaagactgcttgtagtagtcggggatgtcagcggggtgcttgatga
aggccctgctgccgtacataaactgtggagacaggatgtcccagctgaaaggcagagggccgcctttggtcactttcagcttggcggt
ctgagttccctcgtaaggtctgccctcgccttcgccttcgatctcgaactcgtggccgttcatgctgccttccatgtgcaccttgaacctc
atgaactctttgatcacggcctcgcctttagaaaccatggtggcggcccctatagtgagtcgtattattaatcgcgtataagacaaaagt
gttgtggaattgctccaggcgatctgacggttcactaaacgagctctgcttttataggcgcccaccgtacacgcctaaagcttatacgtt
ctctatcactgatagggagtaaactggatatacgttctctatcactgatagggagtaaactgtagatacgttctctatcactgataggga
gtaaactggtcatacgttctctatcactgatagggagtaaactccttatacgttctctatcactgatagggagtaaagtctgcatacgttc
tctatcactgatagggagtaaactcttcatacgttctctatcactgatagggagtaaactcgaggaatacttgaagtcgaaagaagaga
aatgttctggcacctgcacttgcactggggacagcctattttgctagtttgttttgtttcgttttgttttgatggagagcgtatgttaatcg
attcacacaaaaaaccaacacactattgcaatgaaaataaatttccttttatttaaatggccggcctaaaagttttgttactttatagaaga
aattttgagtttttgttttttttttaataaataaataaacataaataaattgtttgttgaatttattattagtatgtaagtgtaaatataataaa
acttaatatctattcaaattaataaataaacctcgatatacagaccgataaaacacatgcgtcaattttacgcatgattatctttaacgta
cgtcacaatatgattatctttctagggttaagtatacacgcgctcactggccgtcgttttacaacgtcgtgactgggaaaaccctggcgt
tacccaacttaatcgccttgcagcacatccccctttcgccagctggcgtaatagcgaagaggcccgcaccgatcgcccttcccaacagt
tgcgcagcctgaatggcgaatgggacgcgccctgtagcggcgcattaagcgcggcgggtgtggtggttacgcgcagcgtgaccgcta
cacttgccagcgccctagcgcccgctcctttcgctttcttcccttcctttctcgccacgttcgccggctttccccgtcaagctctaaatcgg
gggctcccttagggttccgatttagtgctttacggcacctcgaccccaaaaaacttgattagggtgatggttcacgtagtgggccatcg
ccctgatagacggtttttcgccctttgacgttggagtccacgttctttaatagtggactcttgttccaaactggaacaacactcaacccta
tctcggtctattcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgatttaacaaaaatttaacgcgaat
tttaacaaaatattaacgcttacaatttag

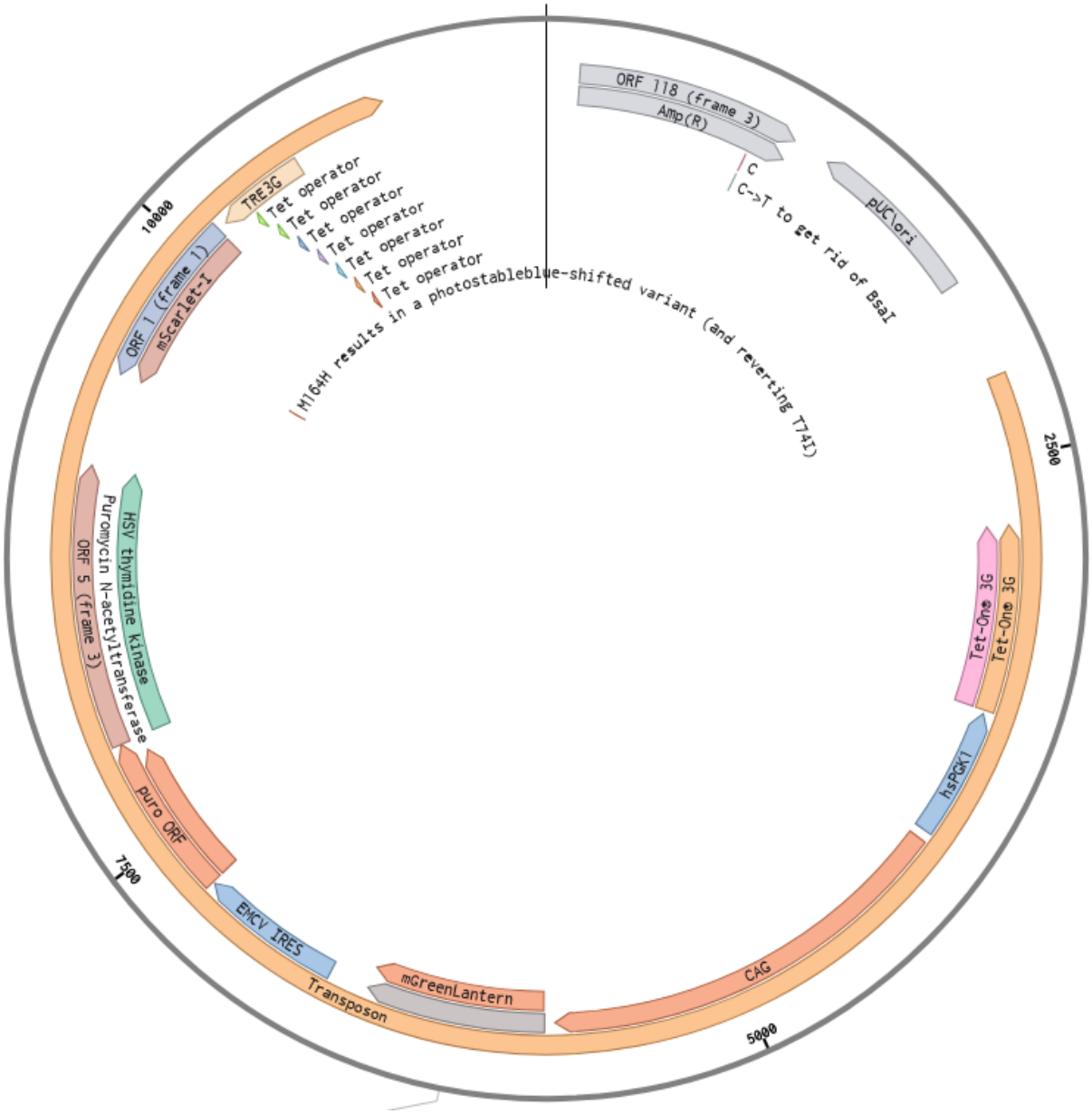


Figure S17: Transposon used for engineering the stable cell line HEK293T which expresses mScarlet-I upon doxycycline addition.

The full sequence of the plasmid encoding the transposase is provided below

ggcgcgcctggtacccgttgaattctaccgggtaggggaggcgcttttcccaaggcagtctggagcatgcgctttagcagccccgctg
ggcacttggcgctacacaagtggcctctggcctcgcacacattccacatccaccggtaggcgccaaccggctccgttctttggtggccc
cttcgcgccaccttctactcctcccctagtcaggaagttcccccccgccccgcagctcgcgtcgtgcaggacgtgacaaatggaagtag
cacgtctcactagtctcgtgcagatggacagcaccgctgagcaatggaagcgggtaggcctttggggcagcggccaatagcagctttg
ctccttcgctttctgggctcagaggctgggaaggggtgggtccgggggcgggctcaggggcgggctcaggggcggggcgggcgccc
gaaggtcctccggaggcccggcattctgcacgcttcaaaagcgcacgtctgccgcgctgttctcctcttcctcatctccgggcctttcga
ccaccggtgatccttaattaataatacgactcactataggggccgccaccatgggcagcagcctggacgacgagcacatcctgagcgc
cctgctgcagagcgacgacgagctggtcggcgaggacagcgacagcgagatcagcgaccacgtgagcgaggacgacgtgcagtcc
gacaccgaggaggccttcatcgacgaggtgcacgaggtgcagcctaccagcagcggctccgagatcctggacgagcagaacgtgat
cgagcagcccggcagctccctggccagcaacaggatcctgaccctgccccagaggaccatcaggggcaagaacaagcactgctggt
ccacctccaagagcaccaggcggagcagggtgtccgccctgaacatcgtgagaagccagaggggccccaccaggatgtgcaggaac
atctacgacccccctgctgtgcttcaagctgttcttcaccgacgagatcatcagcgagatcgtgaagtggaccaacgccgagatcagcct
gaagaggcgggagagcatgaccggcgccaccttcagggacaccaacgaggacgagatctacgccttcttcggcatcctggtgatgac
cgccgtgaggaaggacaaccacatgagcaccgacgacctgttcgacagatccctgagcatggtgtacgtgagcgtgatgagcaggg
acagattcgacttcctgatcagatgcctgaggatggacgacaagagcatcaggcccaccctgcgggagaacgacgtgttcacccccgt
gagaaagatctgggacctgttcatccaccagtgcatccagaactacacccctggcgcccacctgaccatcgacgagcagctgctgggc
ttcaggggcaggtgccccttcaggatgtatatccccaacaagcccagcaagtacggcatcaagatcctgatgatgtgcgacagcggca
ccaagtacatgatcaacggcatgccctacctgggcaggggcacccagaccaacggcgtgcccctgggcgagtactacgtgaaggagc
tgtccaagcccgtccacggcagctgcagaaacatcacctgcgacaactggttcaccagcatccccctggccaagaacctgctgcagga
gccctacaagctgaccatcgtgggcaccgtgagaagcaacaagagagagatccccgaggtcctgaagaacagcaggtccaggcccg
tgggcaccagcatgttctgcttcgacggcccccctgaccctggtgtcctacaagcccaagcccgccaagatggtgtacctgctgtccagc
tgcgacgaggacgccagcatcaacgagagcaccggcaagccccagatggtgatgtactacaaccagaccaagggcggcgtggacac
cctggaccagatgtgcagcgtgatgacctgcagcagaaagaccaacaggtggcccatggccctgctgtacggcatgatcaacatcgc
ctgcatcaacagcttcatcatctacagccacaacgtgagcagcaagggcgagaaggtgcagagccggaaaaagttcatgcggaacct
gtacatgagcctgacctccagcttcatgaggaagaggctggaggccccaccctgaagagatacctgagggacaacatcagcaacat

cctgcccaacgaggtgcccggcaccagcgacgacagcaccgaggagcccgtgatgaagaagaggacctactgcacctactgtccca
gcaagatcagaagaaaggccaacgccagctgcaagaagtgtaagaaggtcatctgccgggagcacaacatcgacatgtgccagagc
tgtttctgaacgcgtaaatgattgcagatccactagttctagagctcgctgatcagcctcgactgtgccttctagttgccagccatctgtt
gtttgcccctcccccgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgaggaaattgcatcgcattgtc
tgagtaggtgtcattctattctgggggggtggggtggggcaggacagcaagggggaggattgggaagagaatagcaggcatgctggg
gatgcggtgggctctatggcttctgaggcggaaagaaccagctggggcgccactggccgtcgttttacaacgtcgtgactgggaaaac
cctggcgttacccaacttaatcgccttgcagcacatccccctttcgccagctggcgtaatagcgaagaggcccgcaccgatcgcccttc
ccaacagttgcgcagcctgaatggcgaatgggacgcgccctgtagcggcgcattaagcgcggcgggtgtggtggttacgcgcagcgt
gaccgctacacttgccagcgccctagcgcccgctcctttcgctttcttcccttcctttctcgccacgttcgccggctttccccgtcaagctc
taaatcgggggctcccctttagggttccgatttagtgctttacggcacctcgaccccaaaaaacttgattagggtgatggttcacgtagtg
ggccatcgccctgatagacggtttttcgccctttgacgttggagtccacgttctttaatagtggactcttgttccaaactggaacaacact
caaccctatctcggtctattcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgatttaacaaaaatttta
acgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttctaa
atacattcaaatatgtatccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaaggaagagtatgagtattcaacat
ttccgtgtcgcccttattccctttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaaga
tcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaa
tgatgagcacttttaaagttctgctatgtggcgcggtattatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactatt
ctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccata
accatgagtgataacactgcggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatggggg
atcatgtaactcgccttgatcgttgggaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaat
ggcaacaacgttgcgcaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatggaggcggataaa
gttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctggagccggtgagcgtgggtctcgcggtatc
attgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtcaggcaactatggatgaacgaaata
gacagatcgctgagataggtgcctcactgattaagcattggtaactgtcagaccaagtttactcatatatactttagattgatttaaaac
ttcatttttaatttaaaaggatctaggtgaagatccttttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcg
tcagaccccgtagaaaagatcaaaggatcttcttgagatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgcta
ccagcggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgt

ccttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggct
gctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggataaggcgcagcggtcgggctgaacgggg
ggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaagcgccacgctt
cccgaagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaac
gcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcctatgg
aaaaacgccagcaacgcggccttttacggttcctggccttttgctggccttttgctcacatgttctttcctgcgttatcccctgattctgt
ggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagc
ggaagagcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaa
gcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttg
tgtggaattgtgagcggataacaatttcacacaggaaacagctatgaccatga

The full sequence of the plasmid encoding the pegRNA is provided below

ataaaacctgcaggcatgcaagcgatcgcggggccgccccccttcaccgagggcctatttcccatgattccttcatatttgcatatacgat
acaaggctgttagagagataattggaattaatttgactgtaaacacaaagatattagtacaaaatacgtgacgtagaaagtaataattt
cttgggtagtttgcagttttaaaattatgttttaaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatat
atcttgtggaaaggacgaaacaccgggcgaagcaggccacgccggtttcagagccaccagaagatatggcttcggtggcaagttgaa
ataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctgtgccctggcccaccttggtcactacactcggatatggcg
tggcctgcttccgcggttctatctagttacgcgttaaaccaactagaatttttactagttctagagcggccccaattcgccctatagtga
gtcgtattacgcgcgctcactggccgtcgttttacaacgtcgtgactgggaaaaccctggcgttacccaacttaatcgccttgcagcac
atccccctttcgccagctggcgtaatagcgaagaggcccgcaccgatcgcccttcccaacagttgcgcagcctgaatggcgaatggga
cgcgccctgtagcggcgcattaagcgcggcgggtgtggtggttacgcgcagcgtgaccgctacacttgccagcgccctagcgcccgct
cctttcgctttcttcccttcctttctcgccacgttcgccggctttccccgtcaagctctaaatcgggggctccctttagggttccgatttagt
gctttacggcacctcgaccccaaaaaacttgattagggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccctttg
acgttggagtccacgttctttaatagtggactcttgttccaaactggaacaacactcaaccctatctcggtctattcttttgatttataagg
gattttgccgatttcggcctattggttaaaaaatgagctgatttaacaaaaatttaacgcgaattttaacaaaatattaacgcttacaatt
taggtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaata
accctgataaatgcttcaataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattccctttttgcggcatttt

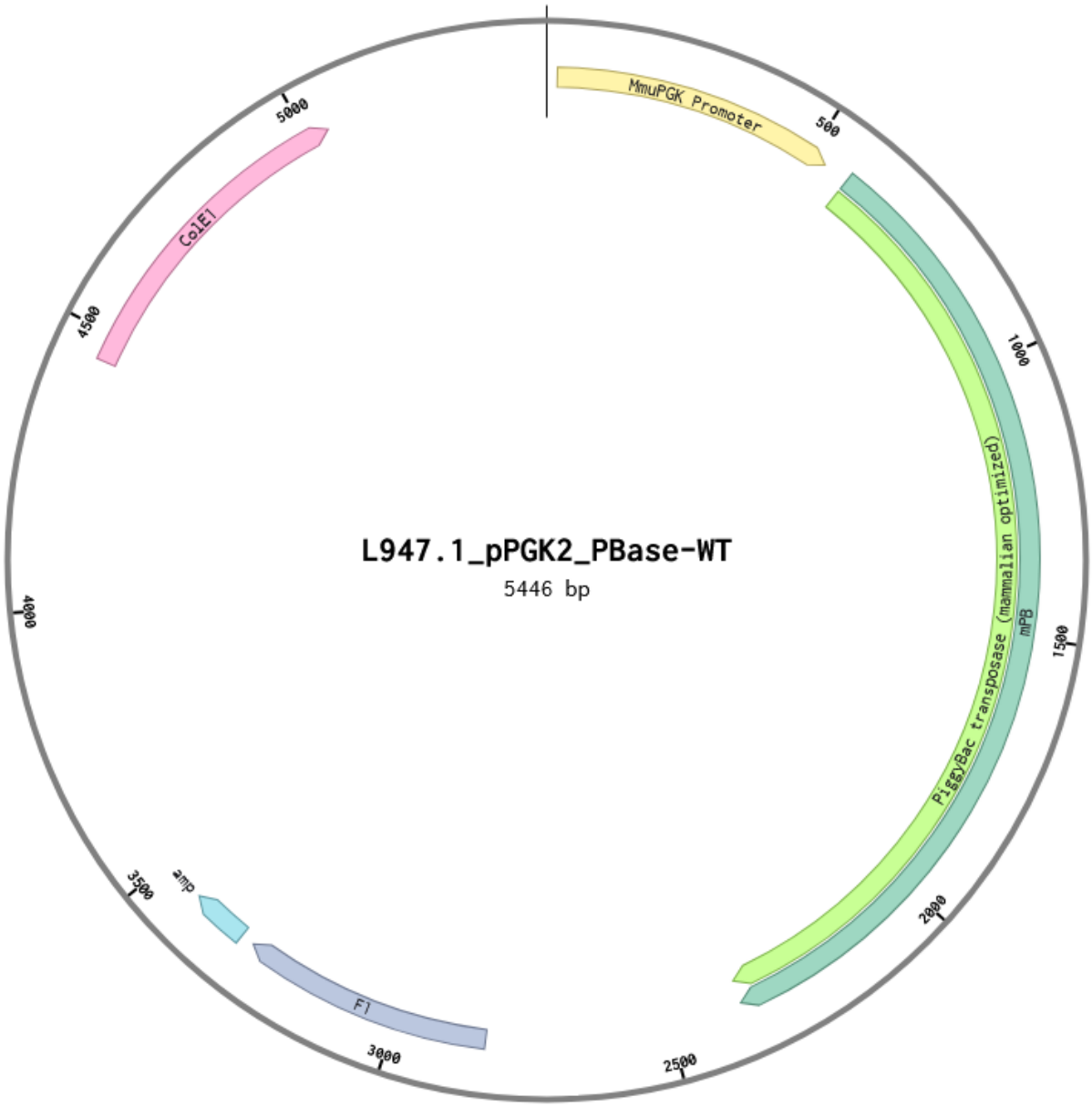


Figure S18: Transposase used for engineering the stable cell line HEK293T which expresses mScarlet-I upon doxycycline addition.

gccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtgggttacatcgaactg
gatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggcgcg
gtattatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcaccagtcac
agaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttactt
ctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccgg
agctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcg
aactacttactctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccg
gctggctggtttattgctgataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctc
ccgtatcgtagttatctacacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgatt
aagcattggtaactgtcagaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagat
ccttttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttct
tgagatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagagcta
ccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtagccgtagttaggccaccacttc
aagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccggg
ttggactcaagacgatagttaccggataaggcgcagcggtcgggctgaacgggggttcgtgcacacagcccagcttggagcgaacg
acctacaccgaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggt
aagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgcca
cctctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggccttttacggttcc
tggccttttgctggccttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgata
ccgctcgccgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcccaatacgcaaaccgcctctcccc
gcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagtt
agctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacag
gaaacagctatgaccatgattacgccaagcgcgcaattaaccctcactaaagggaacaaaagctggaaacatgcatgaagttcctatt
ccgaagttcctattctctagaaagtataggaacttc

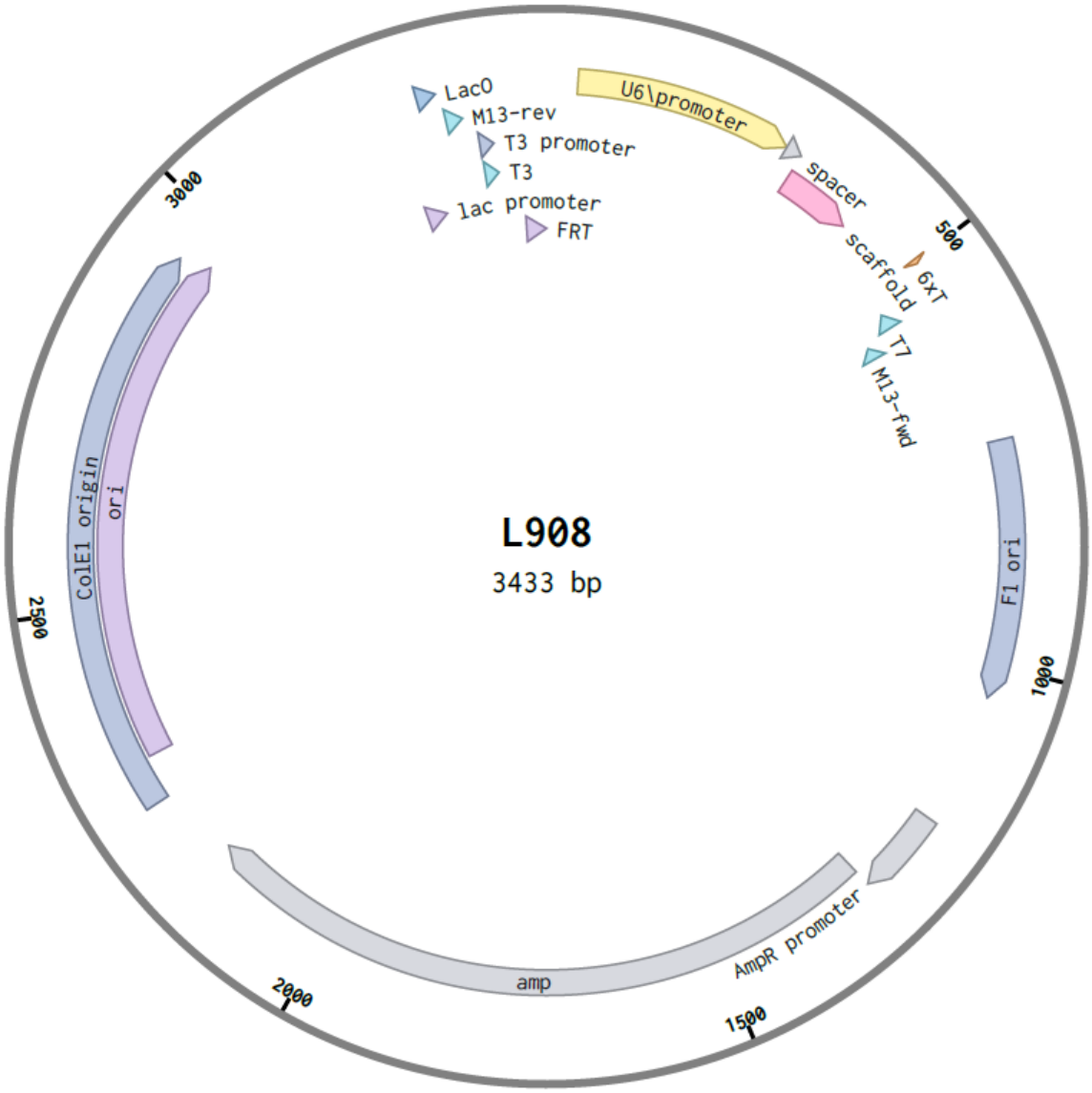


Figure S19: Plasmid used for prime editing experiments. pegRNA is expressed which leads the prime editor.

The full sequence of the plasmid encoding the pegRNA for RNA production is provided below

caattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggata
acaatttcacacaggaaacagctatgaccatgattacgccaagcgcgctaatacgactcactatagggcgaagcaggccacgccggtt
tcagagccaccagaagatatggcttcggtggcaagttgaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgc
tgtgaccaccttaggctacggcgtggcctgcttccgcggttctatctagttacgcgttaaaccaactagaatttttggagaccgcgcgc
tcactggccgtcgttttacaacgtcgtgactgggaaaaccctggcgttacccaacttaatcgccttgcagcacatccccctttcgccagc
tggcgtaatagcgaagaggcccgcaccgatcgcccttcccaacagttgcgcagcctgaatggcgaatgggacgcgccctgtagcggc
gcattaagcgcggcgggtgtggtggttacgcgcagcgtgaccgctacacttgccagcgccctagcgcccgctcctttcgctttcttccct
tcctttctcgccacgttcgccggctttccccgtcaagctctaaatcgggggctccctttagggttccgatttagtgctttacggcacctcg
accccaaaaaacttgattagggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccctttgacgttggagtccacgt
tctttaatagtggactcttgttccaaactggaacaacactcaaccctatctcggtctattcttttgatttataagggattttgccgatttcg
gcctattggttaaaaaatgagctgatttaacaaaaatttaacgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttcg
gggaaatgtgcgcggaaccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataaccctgataaatgctt
caataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgct
cacccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggt
aagatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggcgcggtattatcccgtattg
acgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttac
ggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttacttctgacaacgatcgga
ggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccggagctgaatgaagcca
taccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactacttactctagc
ttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattg
ctgataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatc
tacacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaactgt
cagaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatccttttttgataatctca
tgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatcctttttttct
gcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagagctaccaactctttttccga

aggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtagccgtagttaggccaccacttcaagaactctgtagca
ccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacga
tagttaccggataaggcgcagcggtcgggctgaacgggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactg
agatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggtaagcggcagggtcgg
aacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgt
cgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggccttttacggttcctggccttttgctggcc
ttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagcc
gaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcccaatacgcaaaccgcctctccccgcgcgttggccgatt
cattaatgcagctggcacgacaggtttcccgactggaaagcgggcagtgagcgcaacg

The full sequence of the plasmid encoding the prime editor is provided below

gacattgattattgactagttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgc

The full sequence of the plasmid encoding the prime editor for mRNA production is provided below

gacattgattattgactagttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttac
ggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggg
actttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccct
attgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgta
ttagtcatcgctattaccatggtcgaggtgagccccacgttctgcttcactctccccatctccccccccctccccacccccaattttgtattt
atttatttttaattattttgtgcagcgatgggggcgggggggggggggggcgcgcgccaggcggggcggggcggggcgaggggc
ggggcggggcgaggcggagaggtgcggcggcagccaatcagagcggcgcgctccgaaagtttccttttatggcgaggcggcggcg
gcggcggccctataaaaagcgaagcgcgcggcgggcgggagtcgctgcgtcgcgccttcgccccgtgccccgctccgccgccgcctc
gcgccgcccgccccggctctgactgaccgcgttactcccacaggtgagcgggcgggacggcccttctcctccgggctgtaattagcgc
ttggtttaatgacggctcgtttcttttctgtggctgcgtgaaagccttaaagggctccgggagggccctttgtgcgggggggagcggct

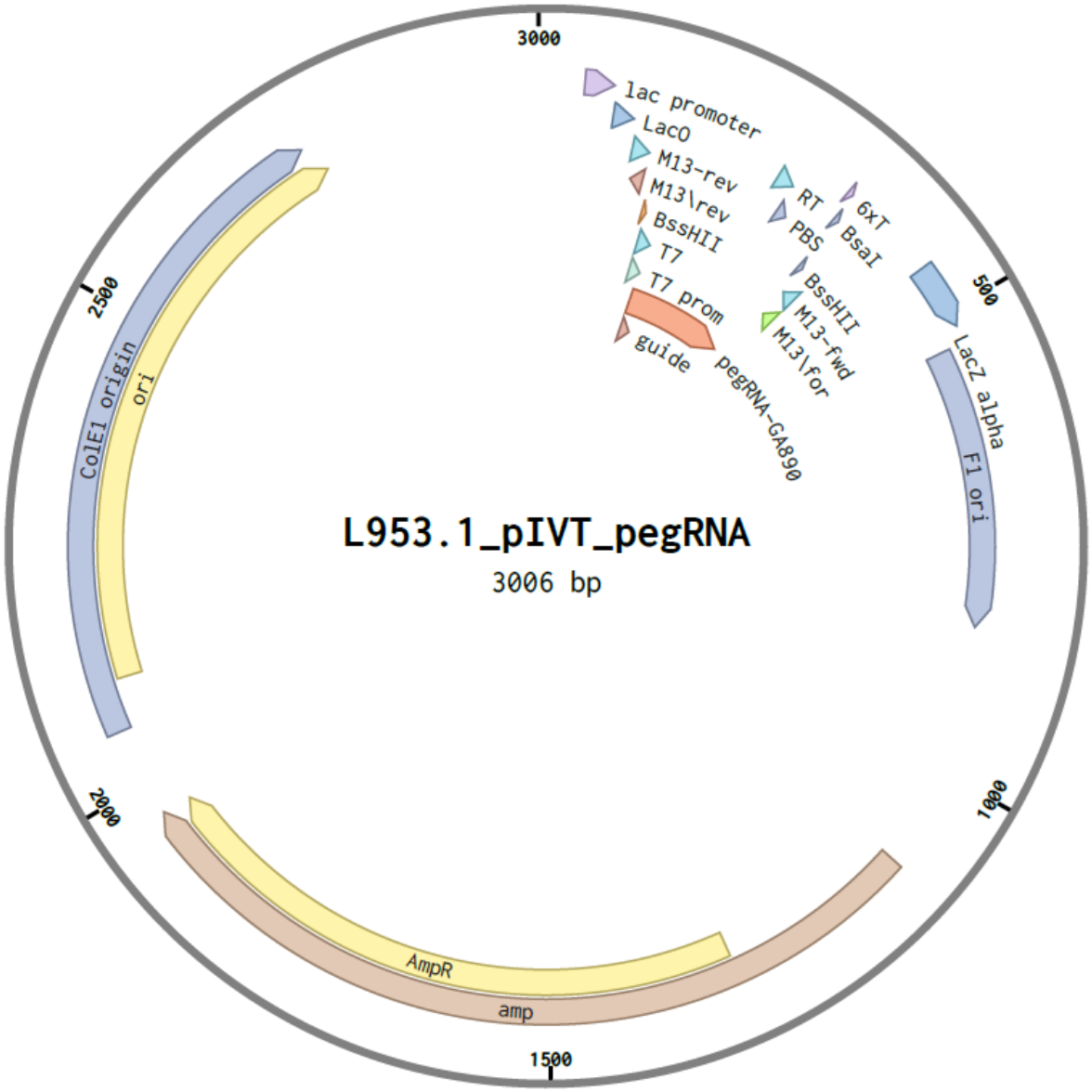


Figure S20: Plasmid used for prime editing experiments. Plasmids used to produce pegRNA via IVT.

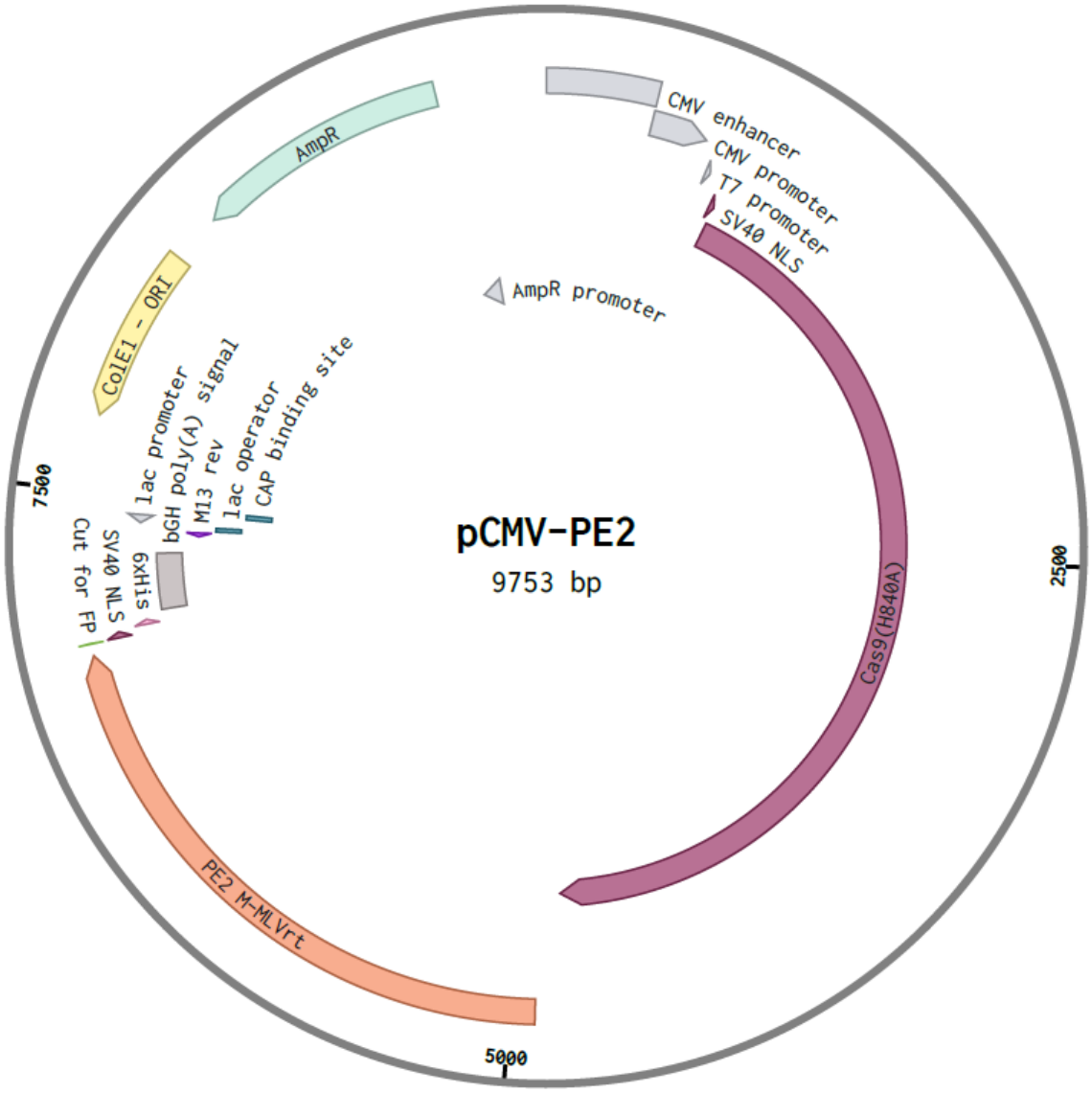


Figure S21: Plasmid used for prime editing experiments. The prime editor is expressed.

cgggggggtgcgtgcgtgtgtgtgtgcgtggggagcgccgcgtgcggcccgcgctgcccggcggctgtgagcgctgcgggcgcggcg

cggggctttgtgcgctccgcgtgtgcgcgaggggagcgcggccgggggcggtgccccgcggtgcgggggggctgcgaggggaaca

aaggctgcgtgcggggtgtgtgcgtgggggggtgagcaggggggtgtgggcgcggcggtcgggctgtaacccccccctgcacccccc

tccccgagttgctgagcacggcccggcttcgggtgcggggctccgtgcggggcgtggcgcggggctcgccgtgccgggcgggggggt

ggcggcaggtgggggtgccgggcggggcggggccgcctcgggccggggagggctcgggggaggggcgcggcggcccccggagcg

ccggcggctgtcgaggcgcggcgagccgcagccattgccttttatggtaatcgtgcgagagggcgcagggacttcctttgtcccaaat

ctggcggagccgaaatctgggaggcgccgccgcaccccctctagcgggcgcgggcgaagcggtgcggcgccggcaggaaggaaat

gggcggggagggccttcgtgcgtcgccgcgccgccgtcccccttctccatctccagcctcggggctgccgcaggggacggctgccttc

ggggggggacggggcagggcggggttcggcttctggcgtgtgaccggcggctctagagcctctgctaaccatgttcatgccttcttctttt

ttcctacagatccttaattaataatacgactcactataaggaatacaagctacttgttctttttgcattgtacaactcactatttgttttcgc

gcccagttgcaaaaagtgtcgccaccatgaccctgaacatcgaggacgagtaccggctgcacgagacaagcaaagaacccgatgtgt

ccctgggcagcacctggcttagtgatttccctcaggcctgggccgagacaggcggaatgggacttgctgttagacaggcccctctgat

catccctctgaaggccacaagcacccctgtgtccatcaagcagtaccccatgagccaagaggcccggctgggaatcaagccccacatt

cagagactgctggaccagggcatcctggtgccttgtcagagcccttggaataccctctgctgcccgtgaagaagcccggcaccaacg

attacagacccgtgcaggacctgcgggaagtgaacaagagagtggaagatattcaccccaccgtgccgaatccttacaacctgctgtc

tggcctgcctcctagccaccagtggtacacagtgctggacctgaaggacgccttcttctgtctgcggctgcaccctacaagccagcctc

tgtttgcctttgagtggcgggaccctgagatgggcattagcggacagctgacctggaccagactgccccagggcttcaagaacagccc

cacactgttcaacgaggccctgcatagggacctcgccgacttcagaatccagcatcctgacctgatcctgctccagtacgtggacgatc

tgctgctggccgctacaagcgagctggattgtcagcagggaacaagagccctgctgcaaaccctgggcaacctgggctatagagcct

ctgccaagaaggcccagatttgccagaaacaagtgaagtatctgggctacctgctgaaagagggccagcgttggctgaccgaggcca

gaaaagaaaccgtgatgggccagcctacacctaagacacccagacagctgagagagttcctgggcaaagccggattctgtcggctgt

tcatccctggctttgccgagatggctgcccctctgtacccactgacaaagcccggaactctgttcaactggggcccagatcagcagaag

gcctaccaagagatcaagcaggctctgctgacagcccctgctctgggactgcctgatctgaccaagcctttcgagctgttcgtggacga

gaagcagggctatgccaaaggcgtgctgacacagaagctcggcccttggagaaggcctgtggcctacctgagcaagaaactggacc

ctgtggctgccggatggcctccttgtctgagaatggtggccgccattgccgtgctgaccaaggatgccggaaagctgacaatgggaca

gcctctggtcattctggcccctcatgccgtggaagccctcgtgaaacagcctcctgatcggtggctgagcaacgccaggatgacacac

tatcaggcactgctgctcgacaccgacagagtgcagtttggacctgtggtggccctgaatcctgccacacttctgcctctgcctgagga

aggcctccagcacaattgcctggacatcctggccgaggctcacggcacaagacccgatctgacagatcagccactgcctgacgccga
ccacacctggtatacagatggcagctctctgctgcaagaaggacagagaaaagccggggctgccgtgaccaccgagacagaagtga
tttgggccaaagctctgcccgctggcacatctgctcagagagccgaactgatcgccctgacacaggccctgaaaatggccgagggca
agaagctgaacgtctacaccgactccagatacgccttcgccaccgctcacatccacggcgaaatctatcggcggagaggatggctgac
cagcgagggcaaagagattaagaacaaggacgagattctcgccctgctcaaggccctgttcctgcctaagcggctgagcatcatccac
tgtccaggccaccagaagggccactctgctgaagctagaggcaacagaatggccgaccaggctgccagaaaggccgccatcacaga
gacacccgataccagcacactgctgatcgagaacagcagccctggatccacgccgcccaagaagaagagaaaggttgaggacggcg
agggcaccggtggacctggaagcgagcctgctacatctggctctgagacacctggcacctccgagtctgctacacctgaatctggacc
tggcggatccggagacaagaagtacagcatcggcctggacatcggcaccaactctgtgggctgggccgtgatcaccgacgagtacaa
ggtgcccagcaagaaattcaaggtgctgggcaacaccgaccggcacagcatcaagaagaacctgatcggagccctgctgttcgacag
cggcgaaacagccgaggccacccggctgaagagaaccgccagaagaagatacaccagacggaagaaccggatctgctatctgcaa
gagatcttcagcaacgagatggccaaggtggacgacagcttcttccacagactggaagagtccttcctggtggaagaggataagaag
cacgagcggcaccccatcttcggcaacatcgtggacgaggtggcctaccacgagaagtaccccaccatctaccacctgagaaagaaa
ctggtggacagcaccgacaaggccgacctgcggctgatctatctggccctggcccacatgatcaagttccggggccacttcctgatcg
agggcgacctgaaccccgacaacagcgacgtggacaagctgttcatccagctggtgcagacctacaaccagctgttcgaggaaaacc
ccatcaacgccagcggcgtggacgccaaggccatcctgtctgccagactgagcaagagcagacggctggaaaatctgatcgcccagc
tgcccggcgagaagaagaatggcctgttcggcaacctgattgccctgagcctgggcctgacccccaacttcaagagcaacttcgacct
ggccgaggatgccaaactgcagctgagcaaggacacctacgacgacgacctggacaacctgctggcccagatcggcgaccagtacg
ccgacctgtttctggccgccaagaacctgtccgacgccatcctgctgagcgacatcctgagagtgaacaccgagatcaccaaggcccc
cctgagcgcctctatgatcaagagatacgacgagcaccaccaggacctgaccctgctgaaagctctcgtgcggcagcagctgcctga
gaagtacaaagagattttcttcgaccagagcaagaacggctacgccggctacattgacggcggagccagccaggaagagttctacaa
gttcatcaagcccatcctggaaaagatggacggcaccgaggaactgctcgtgaagctgaacagagaggacctgctgcggaagcagc
ggaccttcgacaacggcagcatcccccaccagatccacctgggagagctgcacgccattctgcggcggcaggaagatttttacccatt
cctgaaggacaaccgggaaaagatcgagaagatcctgaccttccgcatcccctactacgtgggccctctggccaggggaaacagcag
attcgcctggatgaccagaaagagcgaggaaaccatcacccccctggaacttcgaggaagtggtggacaagggcgcttccgcccaga
gcttcatcgagcggatgaccaacttcgataagaacctgcccaacgagaaggtgctgcccaagcacagcctgctgtacgagtacttcac
cgtgtataacgagctgaccaaagtgaaatacgtgaccgagggaatgagaaagcccgccttcctgagcggcgagcagaaaaaggcca

tcgtggacctgctgttcaagaccaaccggaaagtgaccgtgaagcagctgaaagaggactacttcaagaaaatcgagtgcttcgactc
cgtggaaatctccggcgtggaagatcggttcaacgcctccctgggcacataccacgatctgctgaaaattatcaaggacaaggacttc
ctggacaatgaggaaaacgaggacattctggaagatatcgtgctgaccctgacactgtttgaggacagagagatgatcgaggaacgg
ctgaaaacctatgcccacctgttcgacgacaaagtgatgaagcagctgaagcggcggagatacaccggctggggcaggctgagccg
gaagctgatcaacggcatccgggacaagcagtccggcaagacaatcctggatttcctgaagtccgacggcttcgccaacagaaacttc
atgcagctgatccacgacgacagcctgacctttaaagaggacatccagaaagcccaggtgtccggccagggcgatagcctgcacgag
cacattgccaatctggccggcagccccgccattaagaagggcatcctgcagacagtgaaggtggtggacgagctcgtgaaagtgatg
ggccggcacaagcccgagaacatcgtgatcgaaatggccagagagaaccagaccacccagaagggacagaagaacagccgcgaga
gaatgaagcggatcgaagagggcatcaaagagctgggcagccagatcctgaaagaacaccccgtggaaaacacccagctgcagaa
cgagaagctgtacctgtactacctgcagaatgggcgggatatgtacgtggaccaggaactggacatcaaccggctgtccgactacga
tgtggacgccatcgtgcctcagagctttctgaaggacgactccatcgacaacaaggtgctgaccagaagcgacaagaaccggggcaa
gagcgacaacgtgccctccgaagaggtcgtgaagaagatgaagaactactggcggcagctgctgaacgccaagctgattacccaga
gaaagttcgacaatctgaccaaggccgagagaggcggcctgagcgaactggataaggccggcttcatcaagagacagctggtggaa
acccggcagatcacaaagcacgtggcacagatcctggactcccggatgaacactaagtacgacgagaatgacaagctgatccggga
agtgaaagtgatcaccctgaagtccaagctggtgtccgatttccggaaggatttccagttttacaaagtgcgcgagatcaacaactacc
accacgcccacgacgcctacctgaacgccgtcgtgggaaccgccctgatcaaaaagtaccctaagctggaaagcgagttcgtgtacg
gcgactacaaggtgtacgacgtgcggaagatgatcgccaagagcgagcaggaaatcggcaaggctaccgccaagtacttcttctaca
gcaacatcatgaacttttcaagaccgagattaccctggccaacggcgagatccggaagcggcctctgatcgagacaaacggcgaaa
ccggggagatcgtgtgggataagggccgggattttgccaccgtgcggaaagtgctgagcatgccccaagtgaatatcgtgaaaaaga
ccgaggtgcagacaggcggcttcagcaaagagtctatcctgcccaagaggaacagcgataagctgatcgccagaaagaaggactgg
gaccctaagaagtacggcggcttcgacagccccaccgtggcctattctgtgctggtggtggccaaagtggaaaagggcaagtccaag
aaactgaagagtgtgaaagagctgctggggatcaccatcatggaaagaagcagcttcgagaagaatcccatcgactttctggaagcc
aagggctacaaagaagtgaaaaaggacctgatcatcaagctgcctaagtactccctgttcgagctggaaaacggccggaagagaatg
ctggcctctgccggcgaactgcagaagggaaacgaactggccctgccctccaaatatgtgaacttcctgtacctggccagccactatg
agaagctgaagggctcccccgaggataatgagcagaaacagctgtttgtggaacagcacaagcactacctggacgagatcatcgagc
agatcagcgagttctccaagagagtgatcctggccgacgctaatctggacaaagtgctgtccgcctacaacaagcaccgggataagc
ccatcagagagcaggccgagaatatcatccacctgtttaccctgaccaatctgggagcccctgccgccttcaagtactttgacaccacc

atcgaccggaagaggtacaccagcaccaaagaggtgctggacgccaccctgatccaccagagcatcaccggcctgtacgagacacg

gatcgacctgtctcagctgggaggcgacggatccacaccacctaagaagaaacggaaggtcgaggacggcgagggccctgctgcta

agagagtgaaactggactccggagctgctccagccgccaagaagaagaagctcgactacaaggacgacgacgataagtgaacgcgt

aaatgattgcagatccactagttctagagccaagcacgcagcaatgcagctcaaaacgcttagcctagccacacccccacgggaaac

agcagtgattaacctttagcaataaacgaaagtttaactaagctatactaaccccagggttggtcaatttcgtgccagccacaccctgg

tactgcatgcacgcaatgctagctgccccttttcccgtcctgggtaccccgagtctcccccgacctcgggtcccaggtatgctcccacctc

cacctgccccactcaccacctctgctagttccagacacctccatcgatggcgcgtcttaaataaaaaaaaaaaaaaaaaaaaaaaaa

aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaagcgatcgcgggcggcctctagaggcgcgccgatatcggccgcca

ctggccgtcgttttacaacgtcgtgactgggaaaaccctggcgttacccaacttaatcgccttgcagcacatccccctttcgccagctgg

cgtaatagcgaagaggcccgcaccgatcgcccttcccaacagttgcgcagcctgaatggcgaatgggacgcgccctgtagcggcgca

ttaagcgcggcgggtgtggtggttacgcgcagcgtgaccgctacacttgccagcgccctagcgcccgctcctttcgctttcttcccttcc

tttctcgccacgttcgccggctttccccgtcaagctctaaatcgggggctcccttagggttccgatttagtgctttacggcacctcgacc

ccaaaaaacttgattagggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccctttgacgttggagtccacgttct

ttaatagtggactcttgttccaaactggaacaacactcaaccctatctcggtctattcttttgatttataagggattttgccgatttcggcc

tattggttaaaaaatgagctgatttaacaaaaatttaacgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttcggg

gaaatgtgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataaccctgataaatgcttca

ataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctca

cccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaa

gatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggcgcggtattatcccgtattgac

gccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttacgg

atggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttacttctgacaacgatcggagg

accgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccggagctgaatgaagccata

ccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactacttactctagctt

cccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgct

gataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatcta

cacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaactgtca

gaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatccttttgataatctcatg

accaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatccttttttttctgc
gcgtaatctgctgcttgcaaacaaaaaaaccacccgctaccagcggtggtttgtttgccggatcaagagctaccaactctttttccgaa
ggtaactggcttcagcagagcgcagataccaaatactgttcttctagtgtagccgtagttaggccaccacttcaagaactctgtagcac
cgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgat
agttaccggataaggcgcagcggtcgggctgaacgggggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactg
agatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggtaagcggcagggtcgg
aacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgt
cgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggccttttacggttcctggccttttgctggcc
ttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagcc
gaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcccaatacgcaaaccgcctctccccgcgcgttggccgatt
cattaatgcagctggcacgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattagg
caccccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacaggaaacagctatgacc
atgaggcgcgccggattc

The full sequence of the plasmid encoding the prime editor and an additional mScarlet-I for observation of prime editor expression is provided below

acggcgagatccggaagcggcctctgatcgagacaaacggcgaaaccggggagatcgtgtgggataagggccgggattttgccacc
gtgcggaaagtgctgagcatgccccaagtgaatatcgtgaaaaagaccgaggtgcagacaggcggcttcagcaaagagtctatcctg
cccaagaggaacagcgataagctgatcgccagaaagaaggactgggaccctaagaagtacggcggcttcgacagccccaccgtggc
ctattctgtgctggtggtggccaaagtggaaaagggcaagtccaagaaactgaagagtgtgaaagagctgctggggatcaccatcat
ggaaagaagcagcttcgagaagaatcccatcgactttctggaagccaagggctacaaagaagtgaaaaaggacctgatcatcaagct
gcctaagtactccctgttcgagctggaaaacggccggaagagaatgctggcctctgccggcgaactgcagaagggaaacgaactggc
cctgccctccaaatatgtgaacttcctgtacctggccagccactatgagaagctgaagggctcccccgaggataatgagcagaaacag
ctgtttgtggaacagcacaagcactacctggacgagatcatcgagcagatcagcgagttctccaagagagtgatcctggccgacgcta
atctggacaaagtgctgtccgcctacaacaagcaccgggataagcccatcagagagcaggccgagaatatcatccacctgtttaccct

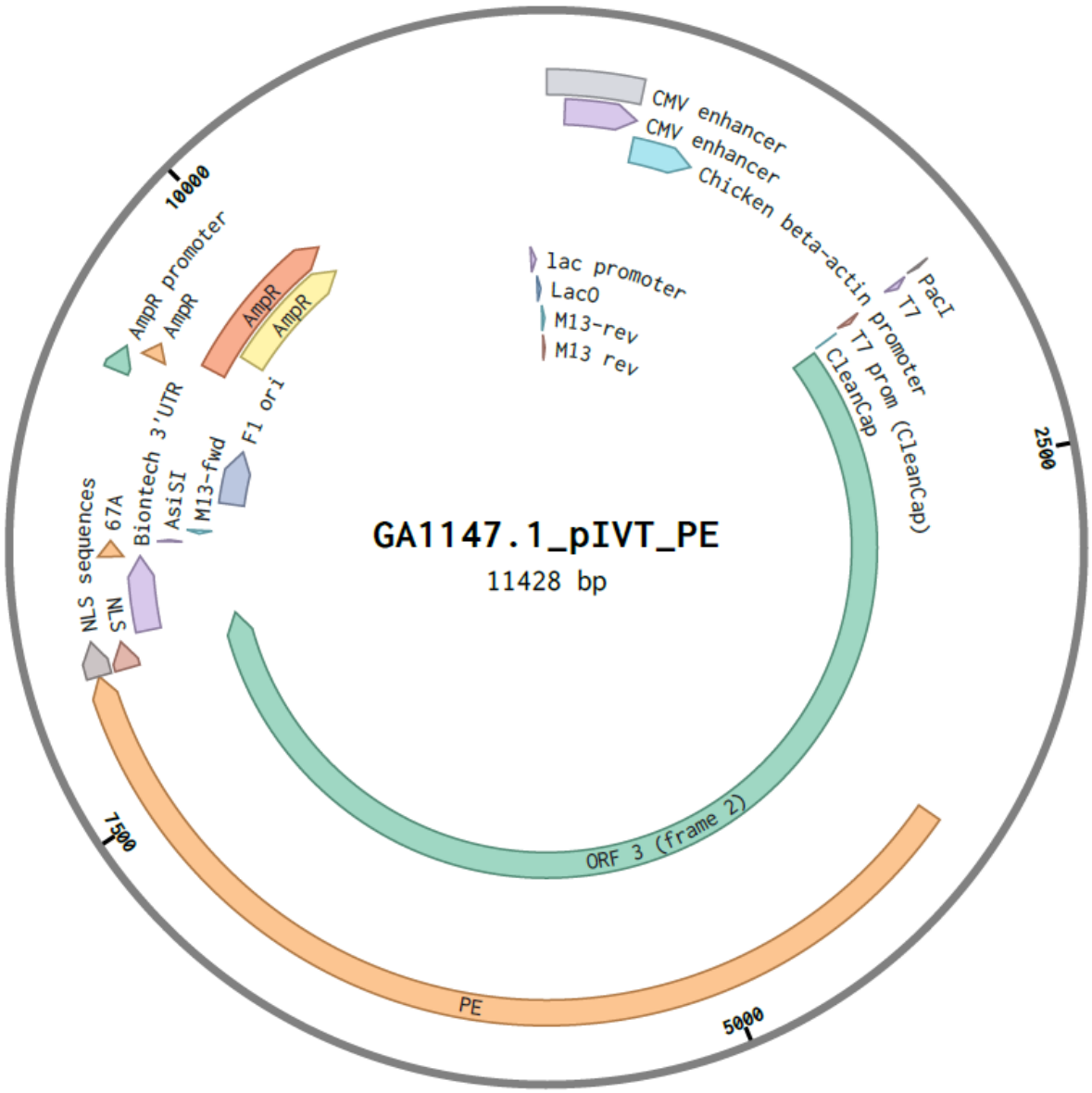


Figure S22: Plasmid used for prime editing experiments. The prime editor and mScarlet-I are expressed to visualize expression of the prime editor.

gaccaatctgggagcccctgccgccttcaagtactttgacaccaccatcgaccggaagaggtacaccagcaccaaagaggtgctgga

cgccaccctgatccaccagagcatcaccggcctgtacgagacacggatcgacctgtctcagctgggaggtgactctggaggatctagc

ggaggatcctctggcagcgagacaccaggaacaagcgagtcagcaacaccagagagcagtggcggcagcagcggcggcagcagca

ccctaaatatagaagatgagtatcggctacatgagacctcaaaagagccagatgtttctctagggtccacatggctgtctgattttcctc

aggcctgggcggaaaccgggggcatgggactggcagttcgccaagctcctctgatcatacctctgaaagcaacctctacccccgtgtc

cataaaacaataccccatgtcacaagaagccagactggggatcaagccccacatacagagactgttggaccagggaatactggtacc

ctgccagtccccctggaacacgcccctgctacccgttaagaaaccagggactaatgattataggcctgtccaggatctgagagaagtc

aacaagcgggtggaagacatccaccccaccgtgcccaacccttacaacctcttgagcgggctcccaccgtcccaccagtggtacactg

tgcttgatttaaaggatgccttttctgcctgagactccaccccaccagtcagcctctcttcgcctttgagtggagagatccagagatgg

gaatctcaggacaattgacctggaccagactcccacagggtttcaaaaacagtcccaccctgtttaatgaggcactgcacagagacct

agcagacttccggatccagcacccagacttgatcctgctacagtacgtggatgacttactgctggccgccacttctgagctagactgcc

aacaaggtactcgggccctgttacaaaccctagggaacctcgggtatcgggcctcggccaagaaagcccaaatttgccagaaacagg

tcaagtatctggggtatcttctaaaagagggtcagagatggctgactgaggccagaaaagagactgtgatggggcagcctactccga

agacccctcgacaactaagggagttcctagggaaggcaggcttctgtcgcctcttcatccctgggtttgcagaaatggcagccccccctg

taccctctcaccaaaccggggactctgtttaattggggcccagaccaacaaaaggcctatcaagaaatcaagcaagctcttctaactgc

cccagccctggggttgccagatttgactaagccctttgaactctttgtcgacgagaagcagggctacgccaaaggtgtcctaacgcaa

aaactgggaccttggcgtcggccggtggcctacctgtccaaaaagctagacccagtagcagctgggtggcccccttgcctacggatgg

tagcagccattgccgtactgacaaaggatgcaggcaagctaaccatgggacagccactagtcattctggcccccccatgcagtagaggc

actagtcaaacaacccccccgaccgctggctttccaacgcccggatgactcactatcaggccttgcttttggacacggaccgggtccagt

tcggaccggtggtagccctgaacccggctacgctgctcccactgcctgaggaagggctgcaacacaactgccttgatatcctggccga

agcccacggaacccgacccgacctaacggaccagccgctcccagacgccgaccacacctggtacacggatggaagcagtctcttaca

agagggacagcgtaaggcgggagctgcggtgaccaccgagaccgaggtaatctgggctaaagccctgccagccgggacatccgctc

agcgggctgaactgatagcactcacccaggccctaaagatggcagaaggtaagaagctaaatgtttatactgatagccgttatgctttt

gctactgcccatatccatggagaaatatacagaaggcgtgggtggctcacatcagaaggcaaagagatcaaaaataaagacgagatc

ttggccctactaaaagccctctttctgcccaaaagacttagcataatccattgtccaggacatcaaaagggacacagcgccgaggcta

gaggcaaccggatggctgaccaagcggcccgaaaggcagccatcacagagactccagacacctctaccctcctcatagaaaattcat

caccctctggcggctcaaaaagaaccgccgacggcagcgaattcgagcccaagaagaagaggaaagtcggaagcggagctactaac

ttcagcctgctgaagcaggctggagacgtggaggagaaccctggacctatggatagcaccgaggcagtgatcaaggagttcatgcgg

ttcaaggtgcacatggagggctccatgaacggccacgagttcgagatcgagggcgagggcgagggccgcccctacgagggcaccca

gaccgccaagctgagggtgaccaagggtggcccccctgcccttctcctgggacatcctgtcccctcagttcatgtacggctccagggcct

tcacgaagcaccccgccgacatccccgactactggaagcagtccttccccgagggcttcaagtgggagcgcgtgatgaacttcgagg

acggcggcgccgtgtccgtggcccaggacacctccctggaggacggcaccctgatctacaaggtgaagctccgcggcaccaacttcc

ctcctgacggccccgtaatgcagaagaagacaatgggctgggaagcatccaccgagcggttgtaccccgaggacgtcgtgctgaagg

gcgacattaagatggccctgcgcctgaaggacggcggccgctacctggcggacttcaagaccacctacagggccaagaagcccgtgc

agatgcccggcgccttcaacatcgaccgcaagttggacatcacatcccacaacgaggactacaccgtggtggaacagtacgaacgct

ccgtggcccgccactccaccggcggctccggtggctccttgtacaagtctggtggttctcccaagaagaagaggaaagtctaaccggt

catcatcaccatcaccattgagtttaaacccgctgatcagcctcgactgtgccttctagttgccagccatctgttgtttgcccctccccg

tgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgagaaaattgcatcgcattgtctgagtaggtgtcattc

tattctgggggggtggggtggggcaggacagcaagggggaggattgggaagacaatagcaggcatgctggggatgcggtgggctcta

tggcttctgaggcggaaagaaccagctggggctcgataccgtcgacctctagctagagcttggcgtaatcatggtcatagctgtttcct

gtgtgaaattgttatccgctcacaattccacacaacatacgagccggaagcataaagtgtaaagcctagggtgcctaatgagtgagct

aactcacattaattgcgttgcgctcactgcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaatcggccaacgcgcg

gggagaggcggtttgcgtattgggcgctcttccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcgagcggtatc

agctcactcaaaggcggtaatacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaagg

ccaggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgccccccctgacgagcatcacaaaaatcgacgctcaagtca

gaggtggcgaaacccgacaggactataaagataccaggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccg

cttaccggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcg

ttcgctccaagctgggctgtgtgcacgaacccccccgttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccg

gtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcgaggtatgtaggcggtgctacagagttcttga

agtggtggcctaactacggctacactagaagaacagtatttggtatctgcgctctgctgaagccagttaccttcggaaaaagagttggt

agctcttgatccggcaaacaaaccaccgctggtagcggtggtttttttgtttgcaagcagcagattacgcgcagaaaaaaaggatctca

agaagatcctttgatcttttctacggggtctgacactcagtggaacgaaaactcacgttaagggattttggtcatgagattatcaaaaag

gatcttcacctagatccttttaaattaaaaatgaagttttaaatcaatctaaagtatatatgagtaaacttggtctgacagttaccaatgc

ttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatccatagttgcctgactccccgtcgtgtagataactacgatacggg

agggcttaccatctggccccagtgctgcaatgataccgcgagacccacgctcaccggctccagatttatcagcaataaaccagccagc

cggaagggccgagcgcagaagtggtcctgcaactttatccgcctccatccagtctattaattgttgccgggaagctagagtaagtagtt

cgccagttaatagtttgcgcaacgttgttgccattgctacaggcatcgtggtgtcacgctcgtcgtttggtatggcttcattcagctccgg

ttcccaacgatcaaggcgagttacatgatcccccatgttgtgcaaaaaagcggttagctccttcggtcctccgatcgttgtcagaagta

agttggccgcagtgttatcactcatggttatggcagcactgcataattctcttactgtcatgccatccgtaagatgcttttctgtgactgg

tgagtactcaaccaagtcattctgagaatagtgtatgcggcgaccgagttgctcttgcccggcgtcaatacgggataataccgcgccac

atagcagaactttaaaagtgctcatcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgctgttgagatccagttcg

atgtaacccactcgtgcacccaactgatcttcagcatcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgc

cgcaaaaaagggaataagggcgacacggaaatgttgaatactcatactcttccttttcaatattattgaagcatttatcagggttattg

tctcatgagcggatacatatttgaatgtatttagaaaaataaacaaatagggttccgcgcacatttccccgaaaagtgccacctgacg

tcgacggatcgggagatcgatctcccgatcccctagggtctactctcagtacaatctgctctgatgccgcatagttaagccagtatctgc

tccctgcttgtgtgttggaggtcgctgagtagtgcgcgagcaaaatttaagctacaacaaggcaaggcttgaccgacaattgcatgaa

gaatctgcttagggttaggcgttttgcgctgcttcgcgatgtacgggccagatatacgcgttgacattgattattgactagttattaatag

taatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcctggctgaccgccc

aacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagta

tttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcccg

cctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcg

gttttggcagtacatcaatgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttgtt

ttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggag

gtctatataagcagagctggtttagtgaaccgtcagatccgctagagatccgcggccgctaatacgactcactatagggagagccgcc

accatgaaacggacagccgacggaagcgagttcgagtcaccaaagaagaagcggaaagtcgacaagaagtacagcatcggcctgg

acatcggcaccaactctgtgggctgggccgtgatcaccgacgagtacaaggtgcccagcaagaaattcaaggtgctgggcaacaccg

accggcacagcatcaagaagaacctgatcggagccctgctgttcgacagcggcgaaacagccgaggccacccggctgaagagaacc

gccagaagaagatacaccagacggaagaaccggatctgctatctgcaagagatcttcagcaacgagatggccaaggtggacgacag

cttcttccacagactggaagagtccttcctggtggaagaggataagaagcacgagcggcaccccatcttcggcaacatcgtggacgag

gtggcctaccacgagaagtaccccaccatctaccacctgagaaagaaactggtggacagcaccgacaaggccgacctgcggctgatc

tatctggccctggcccacatgatcaagttccggggccacttcctgatcgagggcgacctgaaccccgacaacagcgacgtggacaagc

tgttcatccagctggtgcagacctacaaccagctgttcgaggaaaaccccatcaacgccagcggcgtggacgccaaggccatcctgtc

tgccagactgagcaagagcagacggctggaaaatctgatcgcccagctgcccggcgagaagaagaatggcctgttcggaaacctgat

tgccctgagcctgggcctgacccccaacttcaagagcaacttcgacctggccgaggatgccaaactgcagctgagcaaggacaccta

cgacgacgacctggacaacctgctggcccagatcggcgaccagtacgccgacctgtttctggccgccaagaacctgtccgacgccatc

ctgctgagcgacatcctgagagtgaacaccgagatcaccaaggcccccctgagcgcctctatgatcaagagatacgacgagcaccac

caggacctgaccctgctgaaagctctcgtgcggcagcagctgcctgagaagtacaaagagattttcttcgaccagagcaagaacggct

acgccggctacattgacggcggagccagccaggaagagttctacaagttcatcaagcccatcctggaaaagatggacggcaccgagg

aactgctcgtgaagctgaacagagaggacctgctgcggaagcagcggaccttcgacaacggcagcatcccccaccagatccacctgg

gagagctgcacgccattctgcggcggcaggaagatttttacccattcctgaaggacaaccgggaaaagatcgagaagatcctgacctt

ccgcatcccctactacgtgggccctctggccaggggaaacagcagattcgcctggatgaccagaaagagcgaggaaaccatcacccc

ctggaacttcgaggaagtggtggacaagggcgcttccgcccagagcttcatcgagcggatgaccaacttcgataagaacctgcccaa

cgagaaggtgctgcccaagcacagcctgctgtacgagtacttcaccgtgtataacgagctgaccaaagtgaaatacgtgaccgaggg

aatgagaaagcccgccttcctgagcggcgagcagaaaaaggccatcgtggacctgctgttcaagaccaaccggaaagtgaccgtga

agcagctgaaagaggactacttcaagaaaatcgagtgcttcgactccgtggaaatctccggcgtggaagatcggttcaacgcctccct

gggcacataccacgatctgctgaaaattatcaaggacaaggacttcctggacaatgaggaaaacgaggacattctggaagatatcgt

gctgaccctgacactgtttgaggacagagagatgatcgaggaacggctgaaaacctatgcccacctgttcgacgacaaagtgatgaa

gcagctgaagcggcggagatacaccggctggggcaggctgagccggaagctgatcaacggcatccgggacaagcagtccggcaag

acaatcctggatttcctgaagtccgacggcttcgccaacagaaacttcatgcagctgatccacgacgacagcctgaccttaaagagg

acatccagaaagcccaggtgtccggccagggcgatagcctgcacgagcacattgccaatctggccggcagccccgccattaagaagg

gcatcctgcagacagtgaaggtggtggacgagctcgtgaaagtgatgggccggcacaagcccgagaacatcgtgatcgaaatggcc

agagagaaccagaccacccagaagggacagaagaacagccgcgagagaatgaagcggatcgaagagggcatcaaagagctgggc

agccagatcctgaaagaacaccccgtggaaaacacccagctgcagaacgagaagctgtacctgtactacctgcagaatgggcgggat

atgtacgtggaccaggaactggacatcaaccggctgtccgactacgatgtggacgctatcgtgcctcagagctttctgaaggacgact

ccatcgacaacaaggtgctgaccagaagcgacaagaaccggggcaagagcgacaacgtgccctccgaagaggtcgtgaagaagat

gaagaactactggcggcagctgctgaacgccaagctgattacccagagaaagttcgacaatctgaccaaggccgagagaggcggcc

tgagcgaactggataaggccggcttcatcaagagacagctggtggaaacccggcagatcacaaagcacgtggcacagatcctggact

cccggatgaacactaagtacgacgagaatgacaagctgatccgggaagtgaaagtgatcaccctgaagtccaagctggtgtccgattt

ccggaaggatttccagttttacaaagtgcgcgagatcaacaactaccaccacgcccacgacgcctacctgaacgccgtcgtgggaacc

gccctgatcaaaaagtaccctaagctggaaagcgagttcgtgtacggcgactacaaggtgtacgacgtgcggaagatgatcgccaag

agcgagcaggaaatcggcaaggctaccgccaagtacttcttctacagcaacatcatgaacttttcaagaccgagattaccctggcca

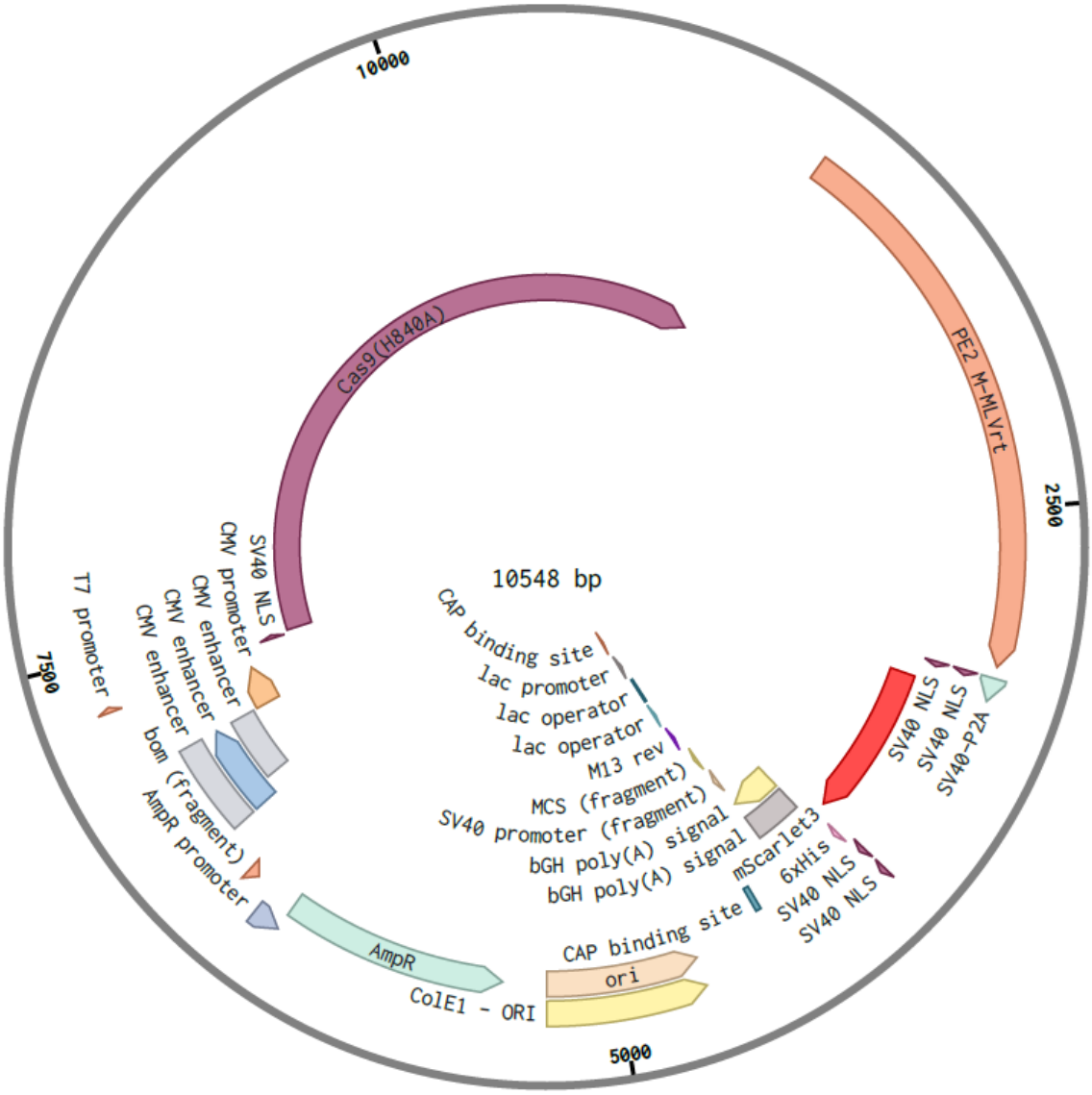


Figure S23: Plasmid used for prime editing experiments. Plasmid used to produce RNA for the prime editor via IVT.

The full sequence of the plasmid encoding the prime editor and an additional mScarlet-I for observation of prime editor expression for mRNA production is provided below

gacattgattattgactagttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttac
ggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaataggg
actttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccct
attgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgta
ttagtcatcgctattaccaggtcgaggtgagccccacgttctgcttcactctccccatctccccccccctccccacccccaattttgtattta
tttatttttaattattttgtgcagcgatgggggcgggggggggggggggcgcgcgccaggcggggcggggcggggcgaggggcg
gggcggggcgaggcggagaggtgcggcggcagccaatcagagcggcgcgctccgaaagtttccttttatggcgaggcggcggcgg
cggcggccctataaaaagcgaagcgcgcggcgggcgggagtcgctgcgcgctgccttcgccccgtgccccgctccgccgccgcctcg
cgccgcccgccccggctctgactgaccgcgttactcccacaggtgagcgggcgggacggcccttctcctccgggctgtaattagcgct
tggtttaatgacggcttgtttcttttctgtggctgcgtgaaagccttgaggggctccgggagggccctttgtgcgggggggagcggctcg
gggggtgcgtgcgtgtgtgtgtgcgtggggagcgccgcgtgcggctccgcgctgcccggcggctgtgagcgctgcgggcgcggcgc
ggggctttgtgcgctccgcagtgtgcgcgaggggagcgcggccgggggcggtgccccgcggtgcgggggggggctgcgaggggaac
aaaggctgcgtgcggggtgtgtgcgtgggggggtgagcaggggggtgtgggcgcgtcggtcgggctgcaaccccccctgcaccccccc
tccccgagttgctgagcacggcccggcttcgggtgcggggctccgtacggggcgtggcgcggggctcgccgtgccgggcgggggggt
ggcggcaggtgggggtgccgggcggggcggggccgcctcgggccggggagggctcgggggaggggcgcggcggcccccggagc
gccggcggctgtcgaggcgcggcgagccgcagccattgccttttatggtaatcgtgcgagagggcgcagggacttcctttgtcccaaa
tctgtgcggagccgaaatctgggaggcgccgccgcacccccctctagcgggcgcggggcgaagcggtgcggcgccggcaggaagga
aatgggcggggagggccttcgtgcgtcgccgcgccgccgtcccccttctccctctccagcctcggggctgtccgcgggggggacggctgc
cttcgggggggacggggcagggcggggttcggcttctggcgtgtgaccggcggctctagagcctctgctaaccatgttcatgccttct
tctttttcctacagatccttaattaataatacgactcactataaggaatacaagctacttgttctttttgcagccgccaccatgaaacgga
cagccgacggaagcgagttcgagtcaccaaagaagaagcggaaagtcgacaagaagtacagcatcggcctggacatcggcaccaa
ctctgtgggctgggccgtgatcaccgacgagtacaaggtgcccagcaagaaattcaaggtgctgggcaacaccgaccggcacagcat
caagaagaacctgatcggagccctgctgttcgacagcggcgaaacagccgaggccacccggctgaagagaaccgccagaagaaga

tacaccagacggaagaaccggatctgctatctgcaagagatcttcagcaacgagatggccaaggtggacgacagcttcttccacaga

ctggaagagtccttcctggtggaagaggataagaagcacgagcggcaccccatcttcggcaacatcgtggacgaggtggcctaccac

gagaagtaccccaccatctaccacctgagaaagaaactggtggacagcaccgacaaggccgacctgcggctgatctatctggccctg

gcccacatgatcaagttccggggccacttcctgatcgagggcgacctgaaccccgacaacagcgacgtggacaagctgttcatccag

ctggtgcagacctacaaccagctgttcgaggaaaaccccatcaacgccagcggcgtggacgccaaggccatcctgtctgccagactg

agcaagagcagacggctggaaaatctgatcgcccagctgcccggcgagaagaagaatggcctgttcggaaacctgattgccctgagc

ctgggcctgacccccaacttcaagagcaacttcgacctggccgaggatgccaaactgcagctgagcaaggacacctacgacgacgac

ctggacaacctgctggcccagatcggcgaccagtacgccgacctgtttctggccgccaagaacctgtccgacgccatcctgctgagcg

acatcctgagagtgaacaccgagatcaccaaggcccccctgagcgcctctatgatcaagagatacgacgagcaccaccaggacctga

ccctgctgaaagctctcgtgcggcagcagctgcctgagaagtacaaagagattttcttcgaccagagcaagaacggctacgccggcta

cattgacggcggagccagccaggaagagttctacaagttcatcaagcccatcctggaaaagatggacggcaccgaggaactgctcgt

gaagctgaacagagaggacctgctgcggaagcagcggaccttcgacaacggcagcatccccccaccagatccacctgggagagctgc

acgccattctgcggcggcaggaagatttttacccattcctgaaggacaaccgggaaaagatcgagaagatcctgaccttccgcatccc

ctactacgtgggccctctggccaggggaaacagcagattcgcctggatgaccagaaagagcgaggaaaccatcacccccctggaactt

cgaggaagtggtggacaagggcgcttccgcccagagcttcatcgagcggatgaccaacttcgataagaacctgcccaacgagaaggt

gctgcccaagcacagcctgctgtacgagtacttcaccgtgtataacgagctgaccaaagtgaaatacgtgaccgagggaatgagaaa

gcccgccttcctgagcggcgagcagaaaaaggccatcgtggacctgctgttcaagaccaaccggaaagtgaccgtgaagcagctgaa

agaggactacttcaagaaaatcgagtgcttcgactccgtggaaatctccggcgtggaagatcggttcaacgcctccctgggcacatac

cacgatctgctgaaaattatcaaggacaaggacttcctggacaatgaggaaaacgaggacattctggaagatatcgtgctgaccctga

cactgtttgaggacagagagatgatcgaggaacggctgaaaacctatgcccacctgttcgacgacaaagtgatgaagcagctgaagc

ggcggagatacaccggctggggcaggctgagccggaagctgatcaacggcatccgggacaagcagtccggcaagacaatcctggat

ttcctgaagtccgacggcttcgccaacagaaacttcatgcagctgatccacgacgacagcctgacctttaaagaggacatccagaaag

cccaggtgtccggccagggcgatagcctgcacgagcacattgccaatctggccggcagccccgccattaagaagggcatcctgcaga

cagtgaaggtggtggacgagctcgtgaaagtgatgggccggcacaagcccgagaacatcgtgatcgaaatggccagagagaaccag

accacccagaagggacagaagaacagccgcgagagaatgaagcggatcgaagagggcatcaaagagctgggcagccagatcctga

aagaacaccccgtggaaaacacccagctgcagaacgagaagctgtacctgtactacctgcagaatgggcgggatatgtacgtggacc

aggaactggacatcaaccggctgtccgactacgatgtggacgctatcgtgcctcagagctttctgaaggacgactccatcgacaacaa

ggtgctgaccagaagcgacaagaaccggggcaagagcgacaacgtgccctccgaagaggtcgtgaagaagatgaagaactactgg

cggcagctgctgaacgccaagctgattacccagagaaagttcgacaatctgaccaaggccgagagaggcggcctgagcgaactgga

taaggccggcttcatcaagagacagctggtggaaacccggcagatcacaaagcacgtggcacagatcctggactcccggatgaacac

taagtacgacgagaatgacaagctgatccgggaagtgaaagtgatcaccctgaagtccaagctggtgtccgatttccggaaggatttc

cagttttacaaagtgcgcgagatcaacaactaccaccacgcccacgacgcctacctgaacgccgtcgtgggaaccgccctgatcaaaa

agtaccctaagctggaaagcgagttcgtgtacggcgactacaaggtgtacgacgtgcggaagatgatcgccaagagcgagcaggaa

atcggcaaggctaccgccaagtacttcttctacagcaacatcatgaacttttttcaagaccgagattaccctggccaacggcgagatccg

gaagcggcctctgatcgagacaaacggcgaaaccggggagatcgtgtgggataagggccgggattttgccaccgtgcggaaagtgc

tgagcatgccccaagtgaatatcgtgaaaaagaccgaggtgcagacaggcggcttcagcaaagagtctatcctgcccaagaggaaca

gcgataagctgatcgccagaaagaaggactgggaccctaagaagtacggcggcttcgacagccccaccgtggcctattctgtgctgg

tggtggccaaagtggaaaagggcaagtccaagaaactgaagagtgtgaaagagctgctggggatcaccatcatggaaagaagcagc

ttcgagaagaatcccatcgactttctggaagccaagggctacaaagaagtgaaaaaggacctgatcatcaagctgcctaagtactccc

tgttcgagctggaaaacggccggaagagaatgctggcctctgccggcgaactgcagaagggaaacgaactggccctgccctccaaa

tatgtgaacttcctgtacctggccagccactatgagaagctgaagggctcccccgaggataatgagcagaaacagctgtttgtggaac

agcacaagcactacctggacgagatcatcgagcagatcagcgagttctccaagagagtgatcctggccgacgctaatctggacaaag

tgctgtccgcctacaacaagcaccgggataagcccatcagagagcaggccgagaatatcatccacctgtttaccctgaccaatctggg

agcccctgccgccttcaagtactttgacaccaccatcgaccggaagaggtacaccagcaccaaagaggtgctggacgccaccctgat

ccaccagagcatcaccggcctgtacgagacacggatcgacctgtctcagctgggaggtgactctggaggatctagcggaggatcctc

tggcagcgagacaccaggaacaagcgagtcagcaacaccagagagcagtggcggcagcagcggcggcagcagcaccctaaatata

gaagatgagtatcggctacatgagacctcaaaagagccagatgtttctctagggtccacatggctgtctgattttcctcaggcctgggc

ggaaaccgggggcatgggactggcagttcgccaagctcctctgatcatacctctgaaagcaacctctacccccgtgtccataaaacaa

taccccatgtcacaagaagccagactggggatcaagccccacatacagagactgttggaccagggaatactggtaccctgccagtccc

cctggaacacgcccctgctacccgttaagaaaccagggactaatgattataggcctgtccaggatctgagagaagtcaacaagcggg

tggaagacatccaccccaccgtgcccaacccttacaacctcttgagcgggctcccaccgtcccaccagtggtacactgtgcttgattta

aaggatgccttttttctgcctgagactccaccccaccagtcagcctctcttcgcctttgagtggagagatccagagatgggaatctcagg

acaattgacctggaccagactcccacagggtttcaaaaacagtcccaccctgtttaatgaggcactgcacagagacctagcagacttc

cggatccagcacccagacttgatcctgctacagtacgtggatgacttactgctggccgccacttctgagctagactgccaacaaggtac

tcgggccctgttacaaaccctagggaacctcgggtatcgggcctcggccaagaaagcccaaatttgccagaaacaggtcaagtatctg

gggtatcttctaaaagagggtcagagatggctgactgaggccagaaaagagactgtgatggggcagcctactccgaagacccctcga

caactaagggagttcctagggaaggcaggcttctgtcgcctcttcatccctgggtttgcagaaatggcagccccccctgtaccctctcac

caaaccggggactctgtttaattggggcccagaccaacaaaaggcctatcaagaaatcaagcaagctcttctaactgccccagccctg

gggttgccagatttgactaagccctttgaactctttgtcgacgagaagcagggctacgccaaaggtgtcctaacgcaaaaactgggac

cttggcgtcggccggtggcctacctgtccaaaaagctagacccagtagcagctgggtggcccccttgcctacggatggtagcagccat

tgccgtactgacaaaggatgcaggcaagctaaccatgggacagccactagtcattctggcccccccatgcagtagaggcactagtcaaa

caaccccccgaccgctggctttccaacgcccggatgactcactatcaggccttgcttttggacacggaccgggtccagttcggaccggt

ggtagccctgaacccggctacgctgctcccactgcctgaggaagggctgcaacacaactgccttgatatcctggccgaagcccacgga

acccgacccgacctaacggaccagccgctcccagacgccgaccacacctggtacacggatggaagcagtctcttacaagagggacag

cgtaaggcgggagctgcggtgaccaccgagaccgaggtaatctgggctaaagccctgccagccgggacatccgctcagcgggctga

actgatagcactcacccaggccctaaagatggcagaaggtaagaagctaaatgtttatactgatagccgttatgcttttgctactgccc

atatccatggagaaatatacagaaggcgtgggtggctcacatcagaaggcaaagagatcaaaaataaagacgagatcttggccctac

taaaagccctctttctgcccaaaagacttagcataatccattgtccaggacatcaaaagggacacagcgccgaggctagaggcaaccg

gatggctgaccaagcggcccgaaaggcagccatcacagagactccagacacctctaccctcctcatagaaaattcatcaccctctggc

ggctcaaaaagaaccgccgacggcagcgaattcgagcccaagaagaagaggaaagtcggaagcggagctactaacttcagcctgct

gaagcaggctggagacgtggaggagaaccctggacctatggatagcaccgaggcagtgatcaaggagttcatgcggttcaaggtgc

acatggagggctccatgaacggccacgagttcgagatcgagggcgagggcgagggccgcccctacgagggcacccagaccgccaa

gctgagggtgaccaagggtggcccccctgcccttctcctgggacatcctgtcccctcagttcatgtacggctccagggccttcacgaagc

accccgccgacatccccgactactggaagcagtccttccccgagggcttcaagtgggagcgcgtgatgaacttcgaggacggcggcg

ccgtgtccgtggcccaggacacctccctggaggacggcaccctgatctacaaggtgaagctccgcggcaccaacttccctcctgacgg

ccccgtaatgcagaagaagacaatgggctgggaagcatccaccgagcggttgtaccccgaggacgtcgtgctgaagggcgacattaa

gatggccctgcgcctgaaggacggcggccgctacctggcggacttcaagaccacctacagggccaagaagcccgtgcagatgcccg

gcgccttcaacatcgaccgcaagttggacatcacatcccacaacgaggactacaccgtggtggaacagtacgaacgctccgtggccc

gccactccaccggcggctccggtggctccttgtacaagtctggtcctgctgctaagagagtgaaactggactgaacgcgtaaatgatt

gcagatccactagttctagagccaagcacgcagcaatgcagctcaaaacgcttagcctagccacacccccacgggaaacagcagtga

ttaacctttagcaataaacgaaagtttaactaagctatactaaccccagggttggtcaatttcgtgccagccacaccctggtactgcatg

cacgcaatgctagctgccccttttcccgtcctgggtaccccgagtctcccccgacctcgggtcccaggtatgctcccacctccacctgccc

cactcaccacctctgctagttccagacacctccatcgatggcgcgtcttaaataaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa

aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaagcgatcgcgggcggcctctagaggcgcgccgatatcggccgccactggccgt

cgttttacaacgtcgtgactgggaaaaccctggcgttacccaacttaatcgccttgcagcacatccccctttcgccagctggcgtaatag

cgaagaggcccgcaccgatcgcccttcccaacagttgcgcagcctgaatggcgaatgggacgcgccctgtagcggcgcattaagcgc

ggcgggtgtggtggttacgcgcagcgtgaccgctacacttgccagcgccctagcgcccgctcctttcgctttcttcccttcctttctcgcc

acgttcgccggctttccccgtcaagctctaaatcgggggctccctttagggttccgatttagtgctttacggcacctcgaccccaaaaaa

cttgattagggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccctttgacgttggagtccacgttctttaatagtg

gactcttgttccaaactggaacaacactcaaccctatctcggtctattcttttgatttataagggattttgccgatttcggcctattggtta

aaaaatgagctgatttaacaaaaatttaacgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttcggggaaatgtgc

gcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataaccctgataaatgcttcaataatattga

aaaaggaagagtatgagtattcaacatttccgtgtcgcccttattccctttttgcggcattttgccttcctgtttttgctcacccagaaac

gctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgag

agttttcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggcgcggtattatcccgtattgacgccgggcaa

gagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttacggatggcatgac

agtaagagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttacttctgacaacgatcggaggaccgaaggag

ctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccggagctgaatgaagccataccaaacgacga

gcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactacttactctagcttcccggcaacaat

taatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctgga

gccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacgggga

gtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaactgtcagaccaagttta

ctcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatccttttgataatctcatgaccaaaatcc

cttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatccttttttctgcgcgtaatctg

ctgcttgcaaacaaaaaaaccacccgctaccagcggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaactggc

ttcagcagagcgcagataccaaatactgttcttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatac

ctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggat

aaggcgcagcggtcgggctgaacgggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctaca

gcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagag
cgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtga
tgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggccttttacggttcctggccttttgctggccttttgctcacat
gttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccga
gcgcagcgagtcagtgagcgaggaagcggaagagcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcag
ctggcacgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggct
ttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacaggaaacagctatgaccatgaggcgcg
ccggattc

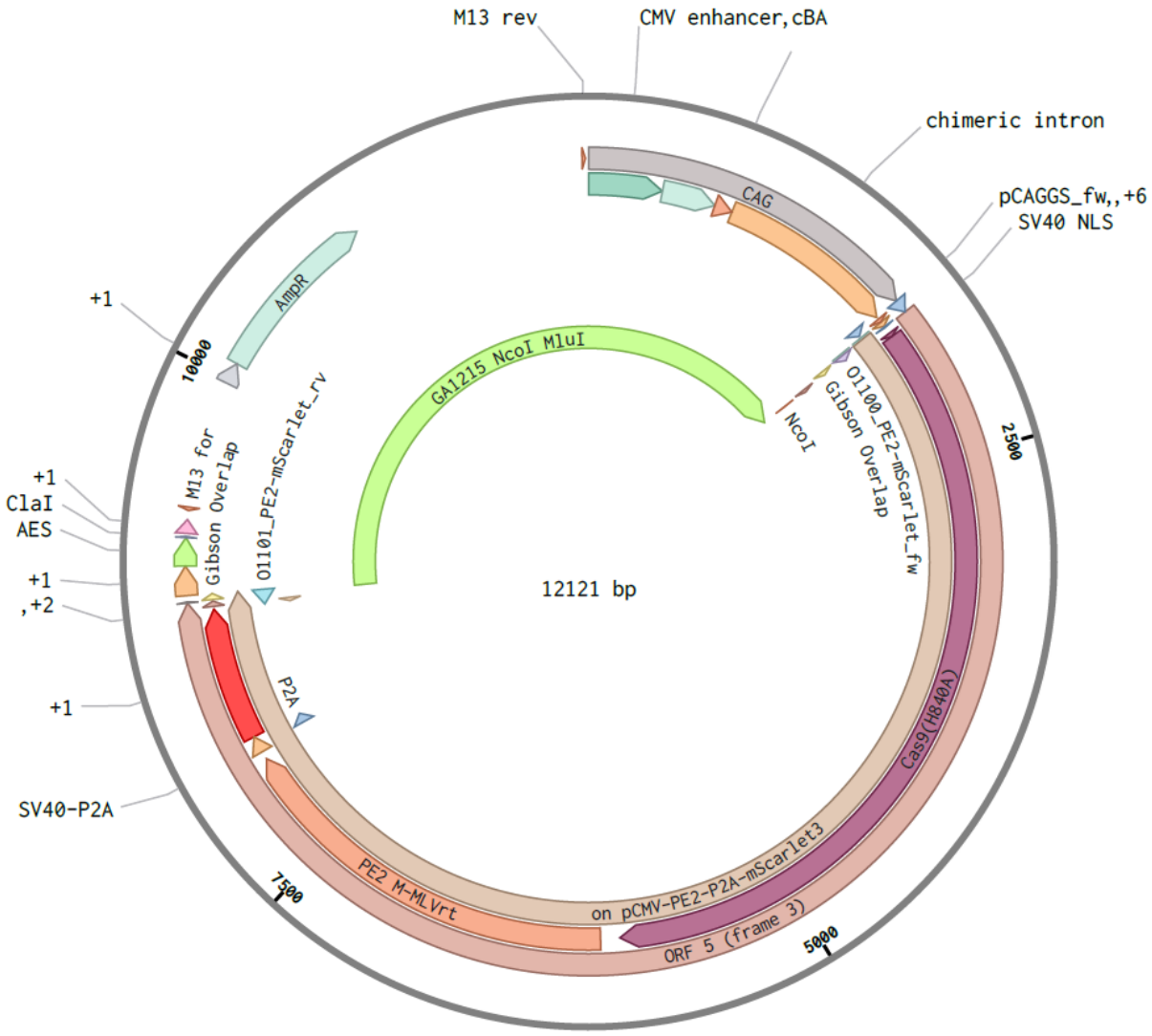


Figure S24: Plasmid used for prime editing experiments. Plasmid used to produce RNA for the prime editor and mScarlet-I for visualization via IVT.

References

1. Liedl, A., Grießing, J., Kretzmann, J. A., and Dietz, H. (2023) Active nuclear import of mammalian cell-expressible DNA origami. *Journal of the American Chemical Society 145*, 4946–4950.