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## Supplemental Information

# Generate TALE/TALEN as Easily and Rapidly as Generating CRISPR

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## Supplementary information

### Reagents used in this study and their manufacturers

The plasmids pIRES2-EGFP, pcDNA-dCas9-VP64, pcDNA-dCas9-VPR, pEGFP-C1, and pEZX-miniCMV-Zsgreen were kept by our laboratory. The Golden Gate TALEN and TAL Effector Kit 2.0 was purchased from Addgene. PrimeSTAR® HS (Premix), Premix Taq™ (TaKaRa Taq™ Version 2.0), DNA Markers, cDNA reverse transcription kit, and some restriction endonucleases, including EcoRI, XhoI, HindIII, and NotI, were purchased from Takara. BsaI, BsmBI, and T4 DNA Ligase were purchased from New England Biolabs (NEB). The plasmid-safe nuclease was purchased from Epicentre Biotechnologies. Lipofectamine™ 2000, Opti-MEM, DAPI, and Trizol were purchased from Invitrogen. The Endo-Free Plasmid kit was purchased from CWBio. Diethylpyrocarbonate (DEPC) was purchased from Sigma. 293T, HepG2, and PANC1 cells were purchased from the Shanghai institutes for biological sciences, Chinese Academy of Sciences (CAS). All oligonucleotides were manufactured by Sangon Biotech (Shanghai). The MinElute Gel Extraction kit and QIAprep Spin Miniprep Kit were purchased from Qiagen. The FastKing RT Kit (with gDNase), the Cell Counting Kit-8 (CCK8) and the competent *E. coli* DH5α were purchased from Tiangen. The fetal bovine serum (FBS) was purchased from HyClone. The Fast SYBR® Green Master Mix was purchased from Applied Biosystems (ABI). The transwell was purchased from Corning. Fibronectin was purchased from Becton Dickinson (BD).

Table S1. Primers for TALE monomers amplification and clony PCR

| Name | Sequence (5′-3′) | Usage |
|---|---|---|
| PCR-TAL-F | TATCATCATGCCTCCTCTAGAG | monomer amplification (universal primers) |
| PCR-TAL-R | TTGGTCATGGGTGGCTCGAGG | |
| PCR-TAL-F1 | TATCATCATGCCTCCTCTAGAGGTCTCCCTATCTTAAAC CGGCCAACATACCCGTCTCCCCCTGAACCTGACCCCG GACCAAGTGGTGGCTATCGCCAGC | First monomer amplification |
| PCR-TAL-R5 | TTGGTCATGGGTGGCTCGAGGGTCTCCATA GAGTCTGTCTTTCCCCTTTCCCGTCTCCTGCACCG | Fifth monomer amplification |
| PCR-TAL-F6 | TATCATCATGCCTCCTCTAGAGGTCTCCCTATCTTAAAC CGGCCAACATACCCGTCTCGTGCAGCGGC | Sixth monomer amplification |
| PCR-TAL-R10 | TTGGTCATGG GTGGCTCGAGGGTCTCCATA GAGTCTGTCTTTCCCCTTTCCCGTCTCCCGCC | Tenth monomer amplification |
| PCR-TAL-R14 | TTGGTCATGG GTGGCTCGAGGGTCTCCTCG AAGTCTGTCTTTCCCCTTTCCCGTCTCCAACAGCCG | Fourteenth monomer amplification |
| PCR-TAL-F15 | TATCATCATGCCTCCTCTAGAGGTCTCCTCGACTTAAAC CGGCCAACATACCCGTCTCCTGTTGCCGG | Fifteenth monomer amplification |
| PCR-TAL-F18 | TATCATCATGCCTCCTCTAGAGGTCTCGCTATCGCCAGC | Eighteenth monomer amplification |
| PCR-TAL-R18 | TTGGTCATGGGTGGCTCGAGGGTCTCTTCGAAGTCTGT CTTTCCCCTTTCCCGTCTCTCGTTGGTCAAC | Eighteenth monomer amplification |
| TAL-F | TTGGCGTCGGCAAACAGTGG | Colony PCR amplification and TALE sequencing |
| TAL-R | GGCGACGAGGTGGTCGTTGG | Colony PCR amplification and TALE sequencing |

Table S2. Oligonucleotides used as templates for amplifying two linker monomers

| Name | Encoded chain sequence (5′-3′) |
|---|---|
| $DNA_{10.5}$ | TATCATCATGCCTCCTCTAGAGGTCTCCTCGACTTAAACCGGCCAACATACCCGTCTCTGGCG GCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCG AGACCCTCGAGCCACCCATGACCAA |
| $DNA_{17.5}$ | TATCATCATGCCTCCTCTAGAGGTCTCCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCC AGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGAGACCCTCGAGCCACCCATGAC CAA |

Table S3. Primers for constructing TALE backbones and fluorescence reporter construct

| Name | Sequence (5′-3′) | Usage |
|---|---|---|
| VP64-F | CCGGAATTCAGCGCTGGAGGAGGTGGAAG | VP64 amplification |
| VP64-R | AAGGAAAAAAGCGGCCGCTCAGTTAATCAGCATGTC | |
| VPR-F | CCGGAATTCGACGCATTGGACGATTTTGATCTGGATAT GCTGGGAAGTG | VPR amplification |
| VPR-R | AAGGAAAAAAGCGGCCGCAAACAGAGATGTGTCGAA GATGGACAGTCCTGTGCTG | |
| HNF4α-F | CCGCTCGAGTGAGATCCAAAACTGAGAC | HNF4α promoter amplification |
| HNF4α-R | CCCAAGCTTAAGCCCACCCAGCCGGAGAG | |
| E47-F | CCGCTCGAGGCTCAGTAGC CACCAACCACC | E47 promoter amplification |
| E47-R | CCCAAGCTTTCTGTGGAG GGGAGCTGGTAAG | |

Table S4. Primers for qPCR detection of gene expression

| Name | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|---|---|---|
| GS | CCTGCTTGTATGCTGGAGTC | GAAAAGTCGTTGATGTTGGA |
| APOCIII | GGGTACTCCTTGTTGTTGC | AAATCCCAGAACTCAGAGAAC |
| CYP1α2 | CTGGCCTCTGCCATCTTCTG | TTAGCCTCCTTGCTCACATGC |
| G-6-P | GGCTCCATGACTGTGGGATC | TTCAGCTGCACAGCCCAGAA |
| PEPCK | GTGTCCCTCTAGTCTATGAAGC | ATTGACTTGATCCTCCAGATAC |
| BR | ACAAGGTGCTGCGGGAATCA | ACTGGTGGGAGGGGTAGGTG |
| ALDOB | AGGAGGACTCTTCTCTCCCAA | GATTCATCTGCAGCCAGGAT |
| APOAI | GCCTTGGGAAAACAGCTAAA | AGGCCCTCTGTCTCCTTTTC |
| GYS2 | CCAGTGGGAAGTCGAAGAAC | TTCTCTCCCCATTCATCTGC |
| HPD | TTGGGAAGGTGAAGTTTGCT | GCATTTGGGCAGTTTAGGAA |
| CD133 | ACATGAAAAGACCTGGGGG | GATCTGGTGT CCCAGCATG |
| CD90 | CGGAAGACCCCAGTCCA | ACGAAGGCTCTGGTCCACTA |
| Oct3/4 | CGACCATCTGCCGCTTTGAG | CCCCCTGTCCCCCATTCCTA |
| SMO | ATCTCCACAGGAGAGACTGGTTCGG | AAAGTGGGGCCTTGGGAACATG |
| β-catenin | AGGTCTGAGGAGCAGCTTCA | ATTGTCCACGCTGGATTTTC |
| Bmi | GGAGACCAGCAAGTATTGTCCTATTT | CATTGTCGCTGGGCATCGTAAG |
| LIN28 | TGTAAGTGGTTCAACGTGCG | CCTCACCCTCCTTCAAGCTC |
| c-Myc | GCTGCTTAGACGCTGGATTT | CTCCTCCTCGTCGCAGTAGA |
| Klf4 | GCGGCAAAACCTACACAAAG | CCCCGTGTGTTTACGGTAGT |
| Sox2 | GCGAACCATCTCTGTGGTCT | GGAAAGTTGGGATCGAACAA |
| ESG1 | GCGCAGTATCACAGCCTTAAA | TCAATCTCTTGGCGATTTCA |
| NANOG | GATTTGTGGGCCTGAAGAAA | TTGGGACTGGTGGAAGAATC |
| β-actin | CCTGGCACCCAGCACAAT | GGGCCGGACTCGTCATACT |
| P21 | GGATGTCCGTCAGAACCCAT | CCCTCCAGTGGTGTCTCGGTG |
| TP53INP1 | AAACCTTCTCATTGAACATCCC | CCATTGTGCTTGACTTGCC |
| HNF6 | AACCCTGGAGCAAACTCAAA | AAGACCAACCTGGGCTTTTT |
| Cx32 | CTGCAGACATTCTCTGGGAAA | GCACCATGATTCTGAAGATGA |
| Top2a | GGGTAGCAATAATCTAAACCT | CCAGTTCTTCAATAGTACCCT |
| Aurka | TCTAGTCCTCCTTAACCACTTATCT | GACACATGGCCTCTTCTGTATC |
| MIST1 | CCAGCACTACCAGCAGCA | AGGACTGGGCGCTAGGTG |
| Sox9 | CACCCGGATTACAAGTACCAG | AAGGTCTCGATGTTGGAGATG |

Table S5. Target sequences of TALENs

| Name | Sequence (5′-3′) | Usage |
|---|---|---|
| Left-HNF4a-TALEN | AATGCTTCCGGGCTGGCA | edit HNF4a |
| Right-HNF4a-TALEN | GGGGCGGGGAGGGCCGAG | |
| Left-E47-TALEN | AACCCCGCCGGGCACATG | edit E47 |
| Right-E47-TALEN | GAAAGCGGGTGGCTCGTC | |

Table S6. Primers amplifying HOM-HNF4a-ZsGreen and HOM-E47-ZsGreen

| Name | Sequence (5′-3′) | Usage |
|---|---|---|
| HOM-HNF4a-F | CTACTGCAGGCTCAAGAAATGCTTCCGGGCTGG CAGGAGGAGGTTCCGGTGGAGGTGGTTCTGGA ATGGCCCAGTCCAAGCACGGCCTGA | HOM-HNF4a-ZsGreen amplification |
| HOM-HNF4a-R | GGTGGGGCAG TGGTGGTGGG GCGGGGAGGG CCGAG TTAGGGCAAGGCGGAGCCGGAGGCG | |
| HOM-E47-F | GCCTGAGCGAAGCCCACAACCCCGCCGGGCAC ATGGGAGGAGGTTCCGGTGGAGGTGGTTCTGG A ATGGCCCAGTCCAAGCACGGCCTGA | HOM-E47-ZsGreen amplification |
| HOM-E47-R | GGCCAGAGCA CAGGGCTGAA AGCGGGTGGC TCGTCTTAGGGCAAGGCGGAGCCGGAGGCG | |

a

Golden Gate TALEN and TAL Effector Kit 2.0 Plate Map

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | pHD1 | pHD2 | pHD3 | pHD4 | pHD5 | pHD6 | pHD7 | pHD8 | pHD9 | pHD10 | pNG1 | pNG2 |
| B | pNG3 | pNG4 | pNG5 | pNG6 | pNG7 | pNG8 | pNG9 | pNG10 | pNI1 | pNI2 | pNI3 | pNI4 |
| C | pNI5 | pNI6 | pNI7 | pNI8 | pNI9 | pNI10 | pNN1 | pNN2 | pNN3 | pNN4 | pNN5 | pNN6 |
| D | pNN8 | pNN8 | pNN9 | pNN10 | pNK1 | pNK2 | pNK3 | pNK4 | pNK5 | pNK6 | pNK7 | pNK8 |
| E | pNK9 | pNK10 | pNH1 | pNH2 | pNH3 | pNH4 | pNH5 | pNH6 | pNH7 | pNH8 | pNH9 | pNH10 |
| F | pLR-HD | pLR-NG | pLR-NI | pLR-NN | pFUS_B1 | pFUS_B2 | pFUS_B3 | pFUS_B4 | pFUS_B5 | pFUS_B6 | pFUS_B7 | pFUS_B8 |
| G | pFUS_B9 | pFUS_B10 | pFUS_A | pFUS_A30A | pFUS_A30B | pLR-NH | pTAL1 | pTAL2 | pTAL3 | pTAL4 | pZHY500 | pZHY501 |
| H | pCP5b | pKEB31 | | | | | | | | | | |

b

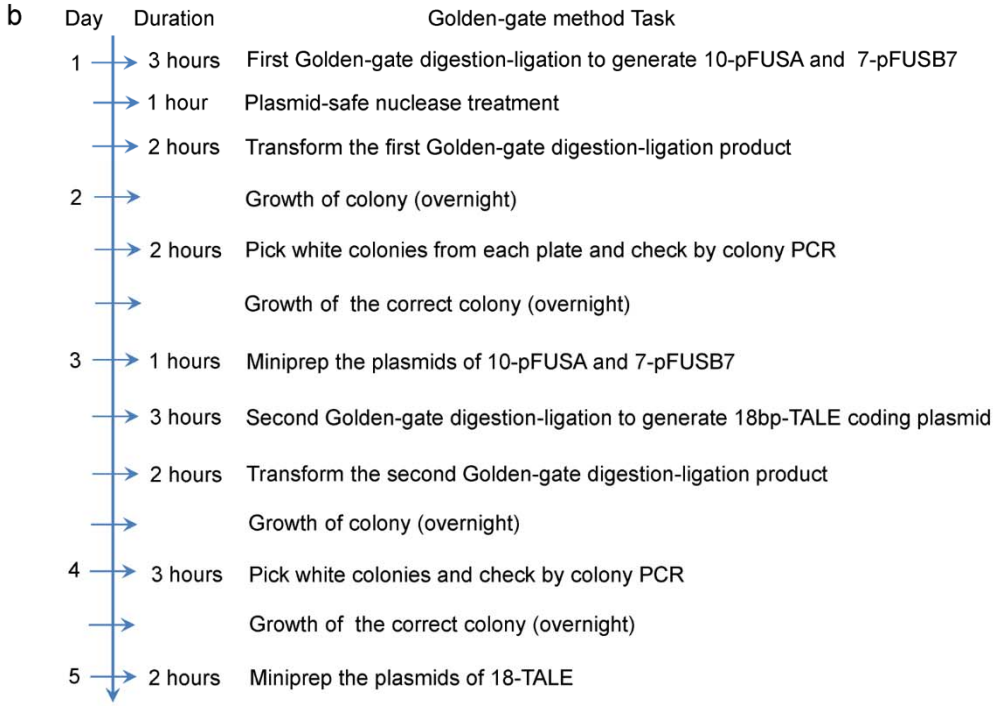



Figure S1. Golden Gate TALEN and TAL Effector Kit 2.0 (Addgene). (a) Plasmid 96-well plate of Addgene kit 2.0. Plasmids were provided as transformed *E.coli* DH5α. (b) Pipeline for constructing TALE with Addgene kit 2.0. This scheme shows an example for constructing a TALE that can bind a target site at the length of 18 bp. 10-pFUSA, a plasmid prepared with 10 monomer plasmids and one receptor plasmid pFUSA in a Golden-gate digestion-ligation reaction; 7-pFUSB7, a plasmid prepared with 7 monomer plasmids and one receptor plasmid pFUSB7 in a Golden-gate digestion-ligation reaction. As a result, 10-pFUSA codes the amino acid binding the N1–N10 nucleotides and 7-pFUSB7 codes the amino acid binding the N11–N17 nucleotides. The nucleotides coding the amino acid binding the last nucleotide (N18) are contained in another monomer plasmid (pLR). The second Golden-gate digestion-ligation reaction contains four plasmids including 10-pFUSA, 7-pFUSB7, pLR, and a TALE backborn vector (pTAL1).

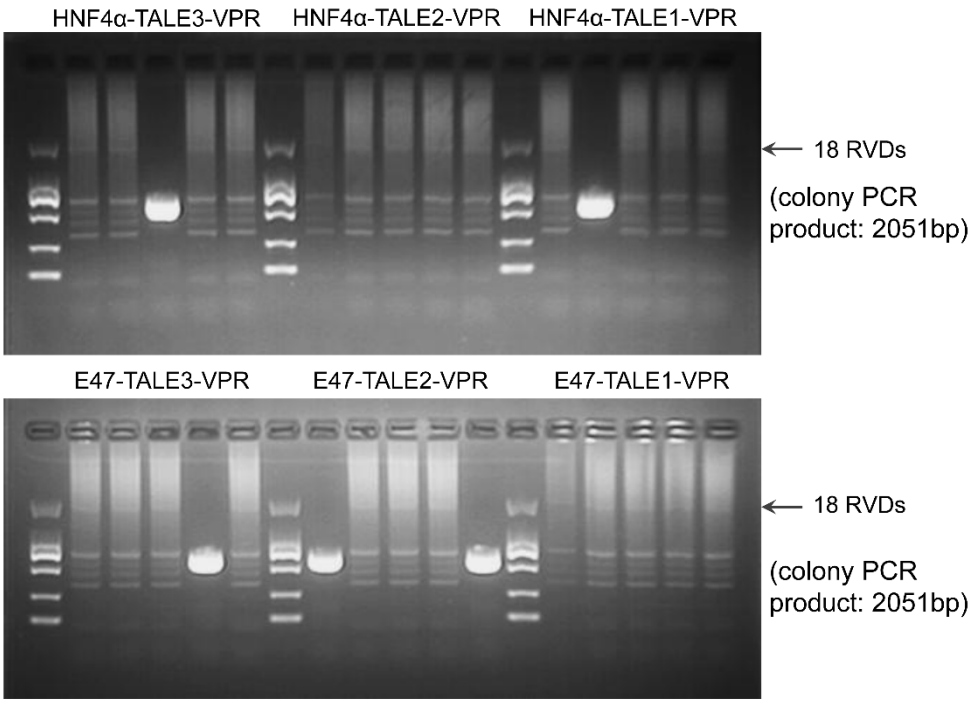



Figure S2. Detection of final TALE-VPR colonies with colony PCR. Full-length PCR products are often less prominent while the "ladder effect" represents a robust indicator of successful assembly. DNA Marker: DL2000 mark. Randomly picked white spots were detected with by colony PCR colony PCR, which indicated that most white spots are correctly assembled TALEs.

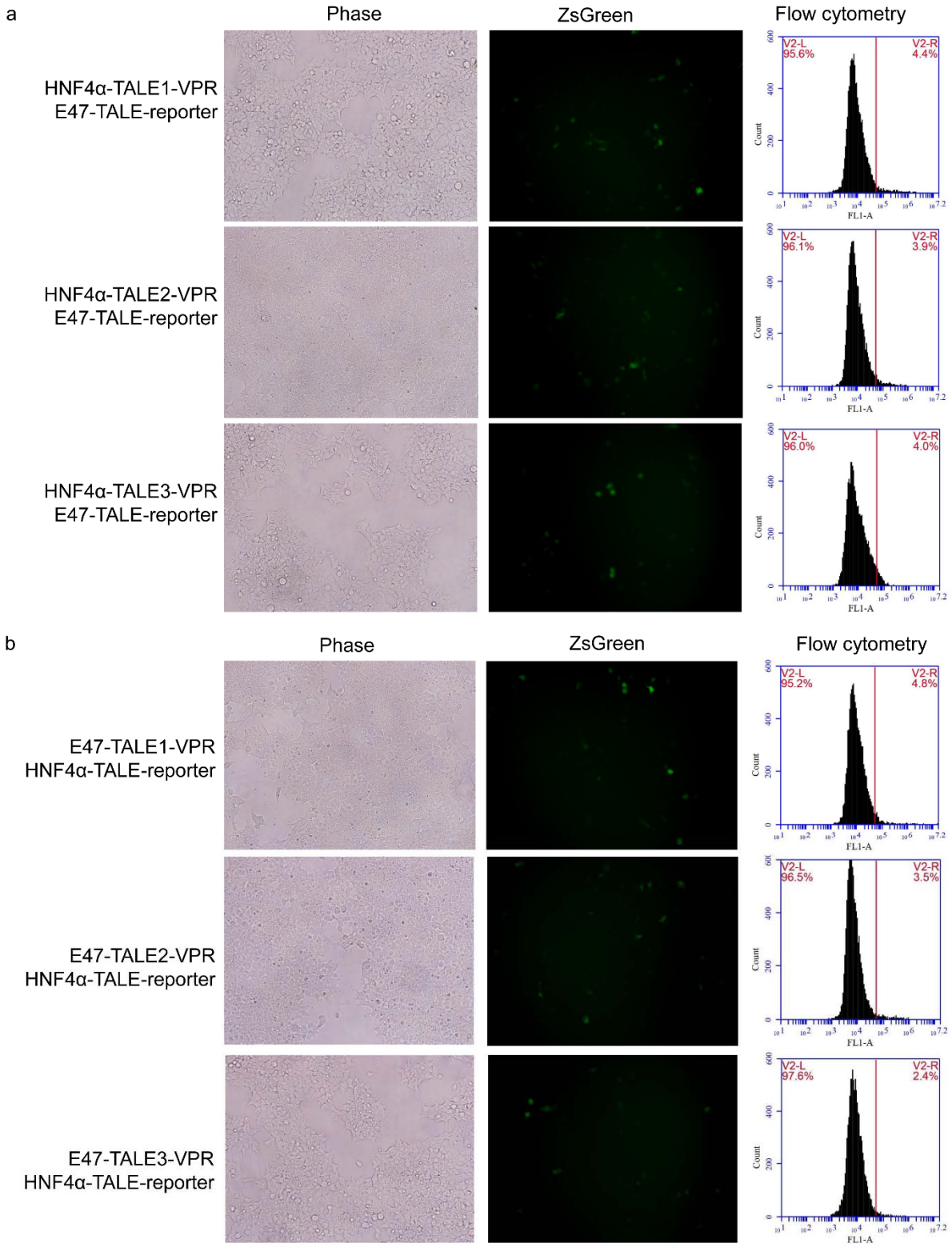



Figure S3. Activating exogenous reporter gene with TALEs. 293T cells were co-transfected with a TALE-VPR plasmid and its unrelated corresponding reporter plasmid expressing ZsGreen. (a) Cells co-transfected with HNF4α-TALE1/2/3-VPR and E47-TALE-reporter. (b) Cells cotransfected with E47-TALE1/2/3-VPR and HNF4α-TALE-reporter.

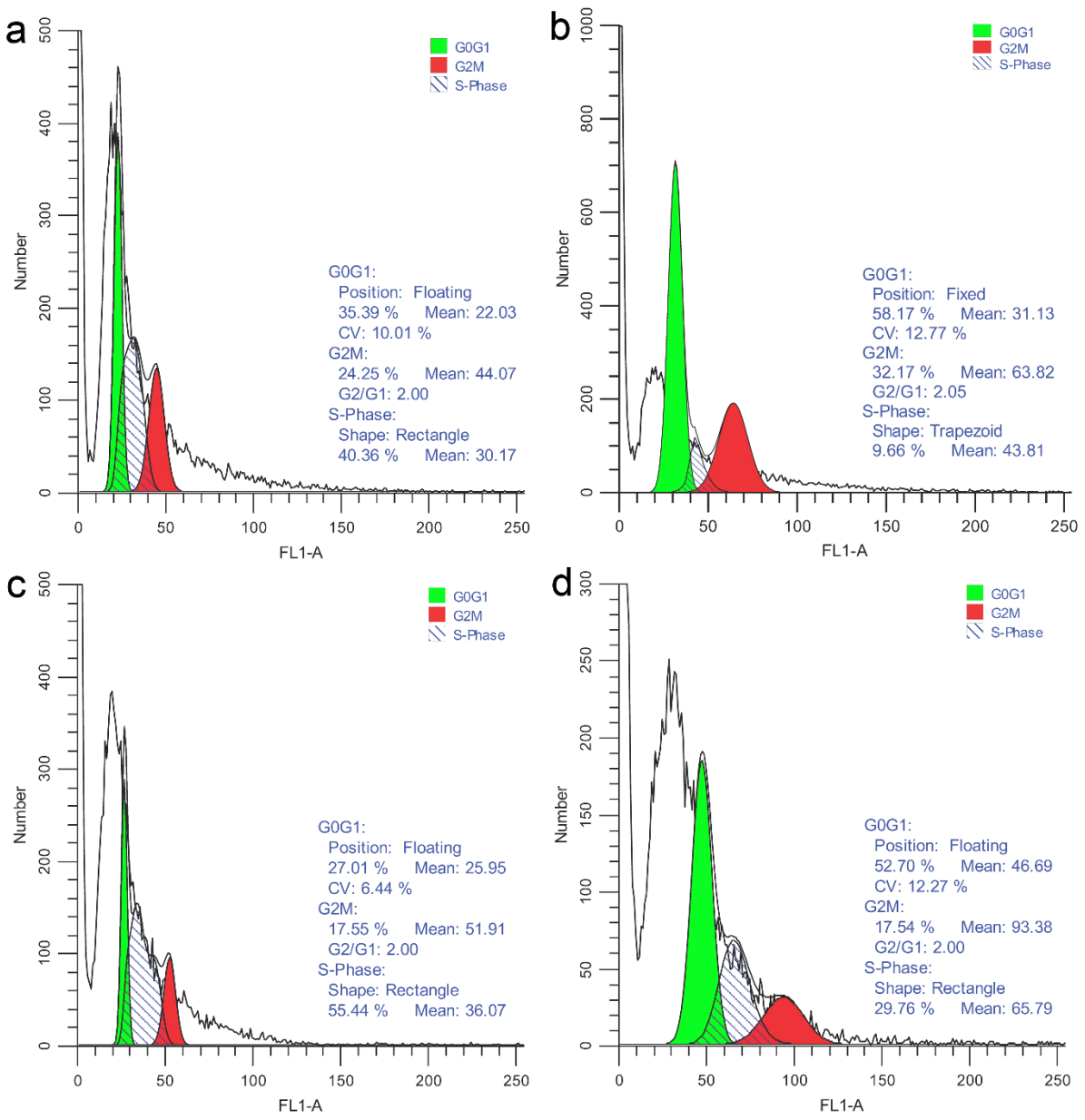
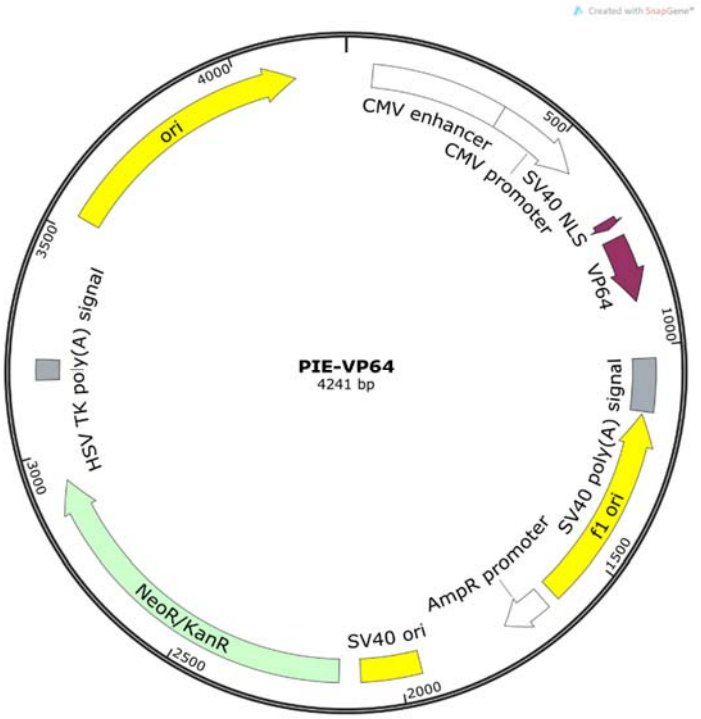
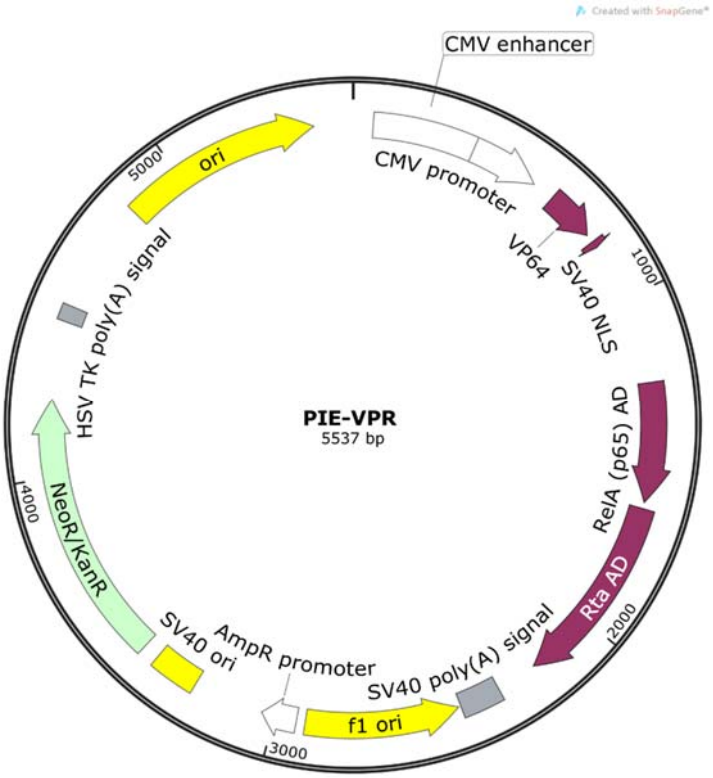
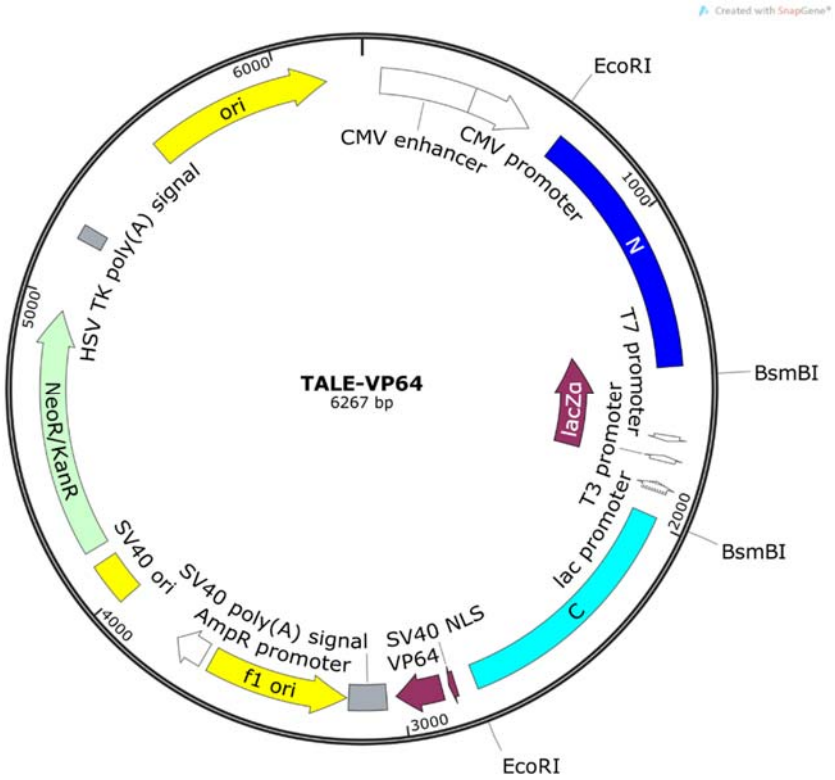



Figure S4. Detection of cell cycle with flow cytometry. (a) HepG2 treated by lipo. (b) HepG2 treated by lipo and HNF4α-TALE1-VPR. (C) PANC1 treated by lipo. (D) PANC1 treated by lipo and E47-TALE1-VPR.

**Vectors and their elements and sequences**






CMV enhancer + CMV promoter + N-term of TALE + Repeating sequence area of TALE instead of LacZa + C-term of TALE+VP64 (SV40NLS+VP64)

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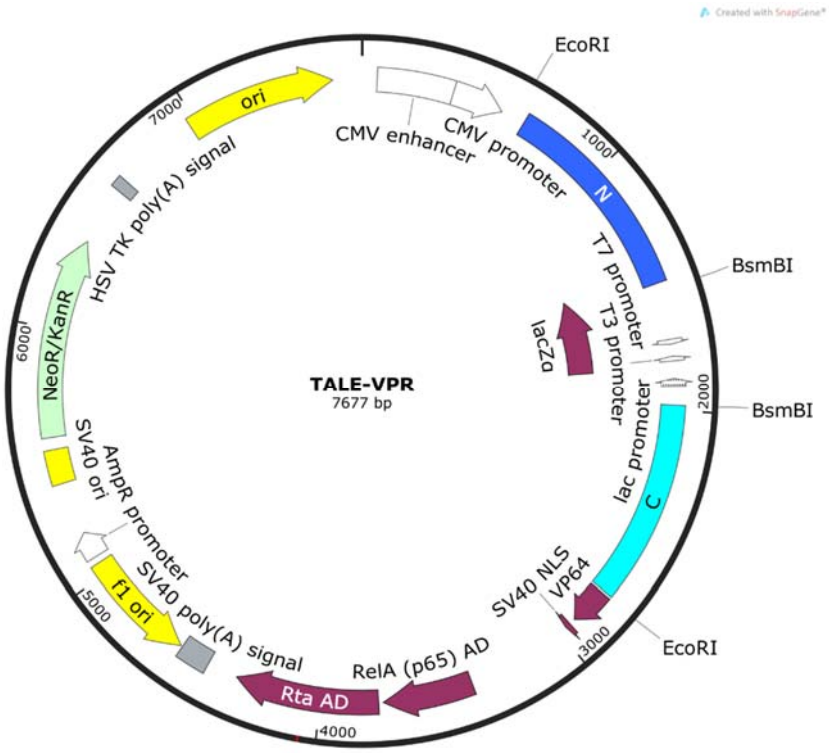


CMV enhancer + CMV promoter + N-term of TALE + Repeating sequence area of TALE instead of LacZa + C-term of TALE + VPR (VP64+SV40 NLS + Rel A(p65)AD + RtaAD)

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TCCCCAGAAGGAAGAGGCTGCAATCTGTGGCCAAATGGACCTTTCCCATCCGCCCCCAAGGGG
CCATCTGGATGAGCTGACAACCACACTTGAGTCCATGACCGAGGATCTGAACCTGGACTCACC
CCTGACCCCGGAATTGAACGAGATTCTGGATACCTTCCTGAACGACGAGTGCCTCTTGCATGCC
ATGCATATCAGCACAGGACTGTCCATCTTCGACACATCTCTGTTTGCGGCCGCGACTCTAGATC

ATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCT
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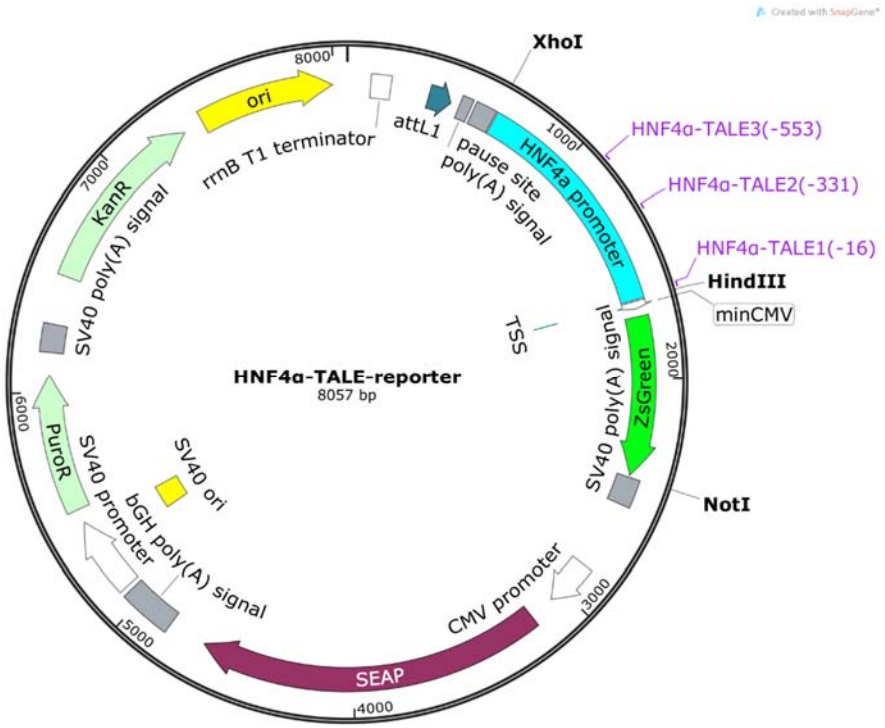


HNF4α promoter+miniCMV+ZSgreen

TALE-binding sites are showedin highlight: TALE1, TALE2, and TALE3

TGAGATCCAAAACTGAGACAAAAGAAACGGGGCTGTTCCAAAAAAAAAGCTAGGTGGCAGG
TGTCTAACATGCCAGGGAGCTAAAACAGAGTGTGTGAGTTTCAGCAGCAGGTTGAATTTAGA
ATGGGGAAGGAGACCAGAGGAGACGCCAGACAGGATGACTTTGTCCCATTGGCCTGGAGGC
AGCCCCATGTTTCTCCACCCCTCATATCACTCACCAGTTTGTAATAGTATCTTTGAATGACGAT
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CCATATCCCCAGAGTCTAGCACAAGGCCTGGTACATAGTAGGTGCTCAATAAATATGTTAGAT
GAAAGGAAGATAACACCTCTATGTACTAGCAGTGAGACTCCAGGCATGCAATTTCTCTCTGTC
CTTCAGTCCCTTCATCTCAAGGTTTAATTTAAATATGGTAACGCCTGTATGCAACTCCCAGCAT
CCAGTAGGCACTCACTAAACACAGTTCTCCACCCTCCTTTTTTCCTCTGCCCCTCCCTCGGTTT
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ATACACCTGGCTCTGGGTTCCCCTAACCCCAGAGTGCAGGACTAGGACCCGAGTGGACCTCA
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CCTTCCTGTTCGAGGACGGCGCCGTGTGCATCTGCAACGCCGACATCACCGTGAGCGTGGAG
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AGGGCATCTTGAAGGGCGACGTGAGCATGTACCTGCTGCTGAAGGACGGTGGCCGCTTGCGC
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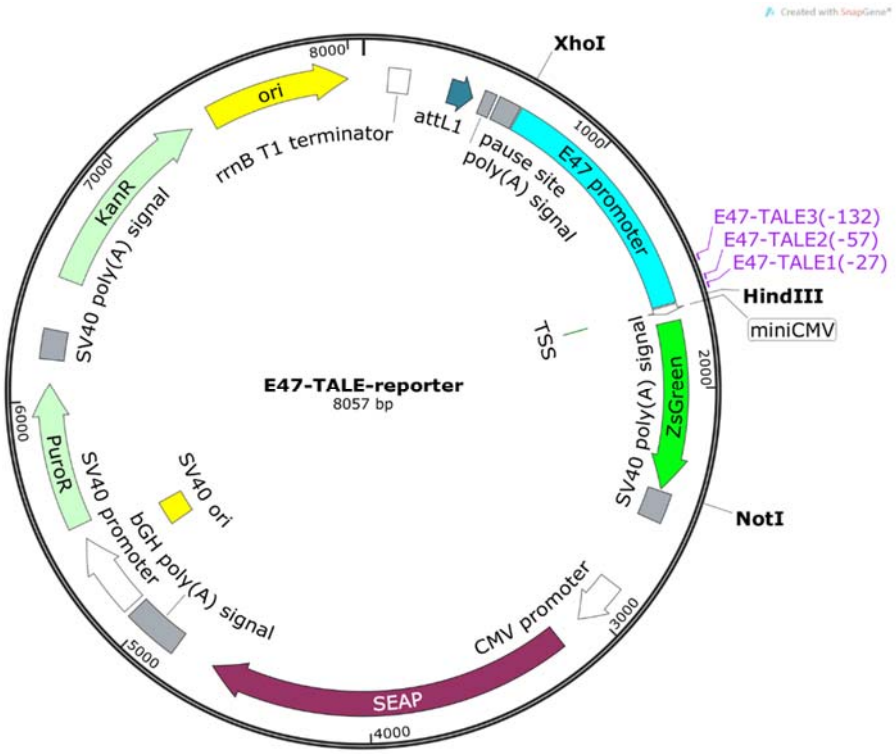


E47 promoter + miniCMV promoter + ZsGreen

TALE-binding sites are showedin highlight: TALE1, TALE2, and TALE3

GCTCAGTAGCCACCAACCACCCTCTGCCCAGTGCCTGTGCTGGCCCCAAGAGCCCTGGGCATA
CATCTCCTCACCCCTCCAGCCGACCAGCTCCCCACAGGCCTGGGTTCGGGGTTAGAGTCAGGT
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CAGGCAGTGCCAACCAGTCAGAGCTGGCGCTACTCACAGACCCCTCTTCCTGTTACTTTAGTG
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GCATGGGCTGATCGTGGTCCCTCCGTCCTGACTGCACCCCCCACCGCCCACCCGCCCGCAGGT
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CCCCCAGCAGCACCTATCCCGCCCCCTTCTACGTGGCAGGTACATGGCAGGGCGGGGGCTCG
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