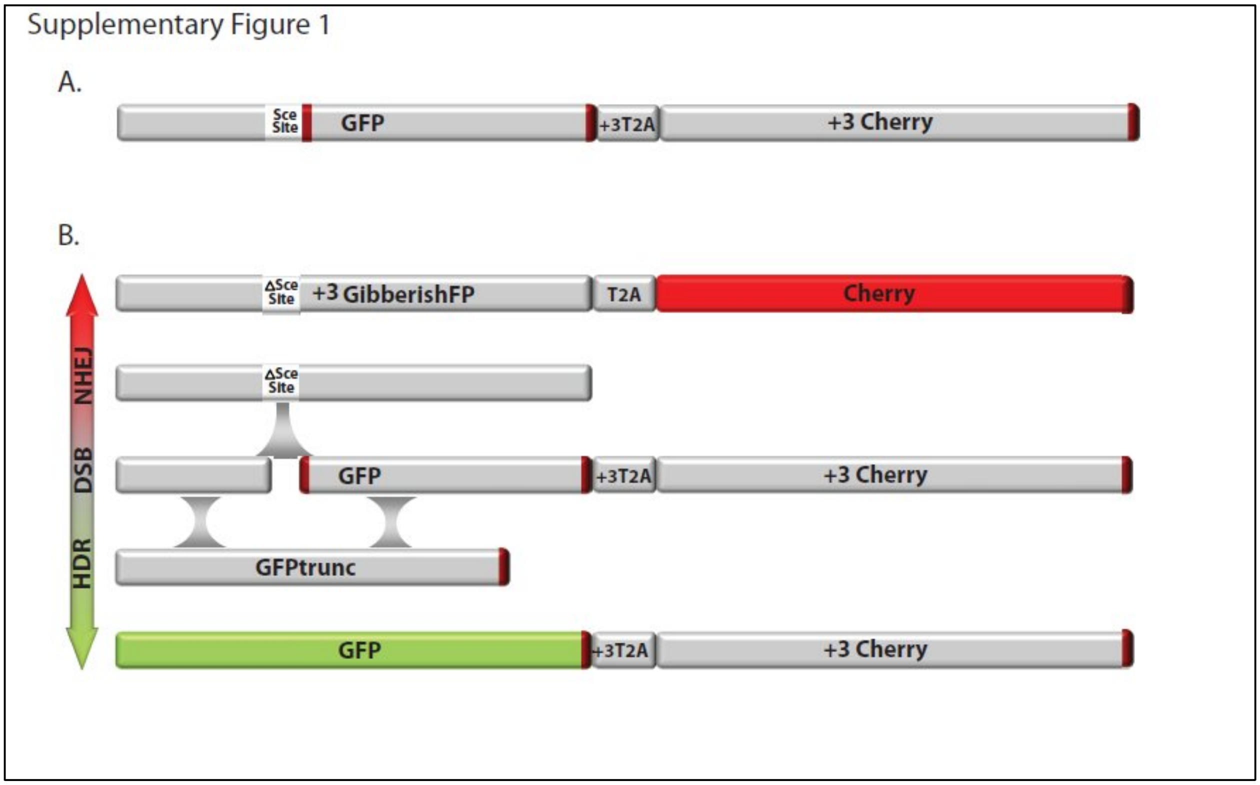
Supplementary Figures

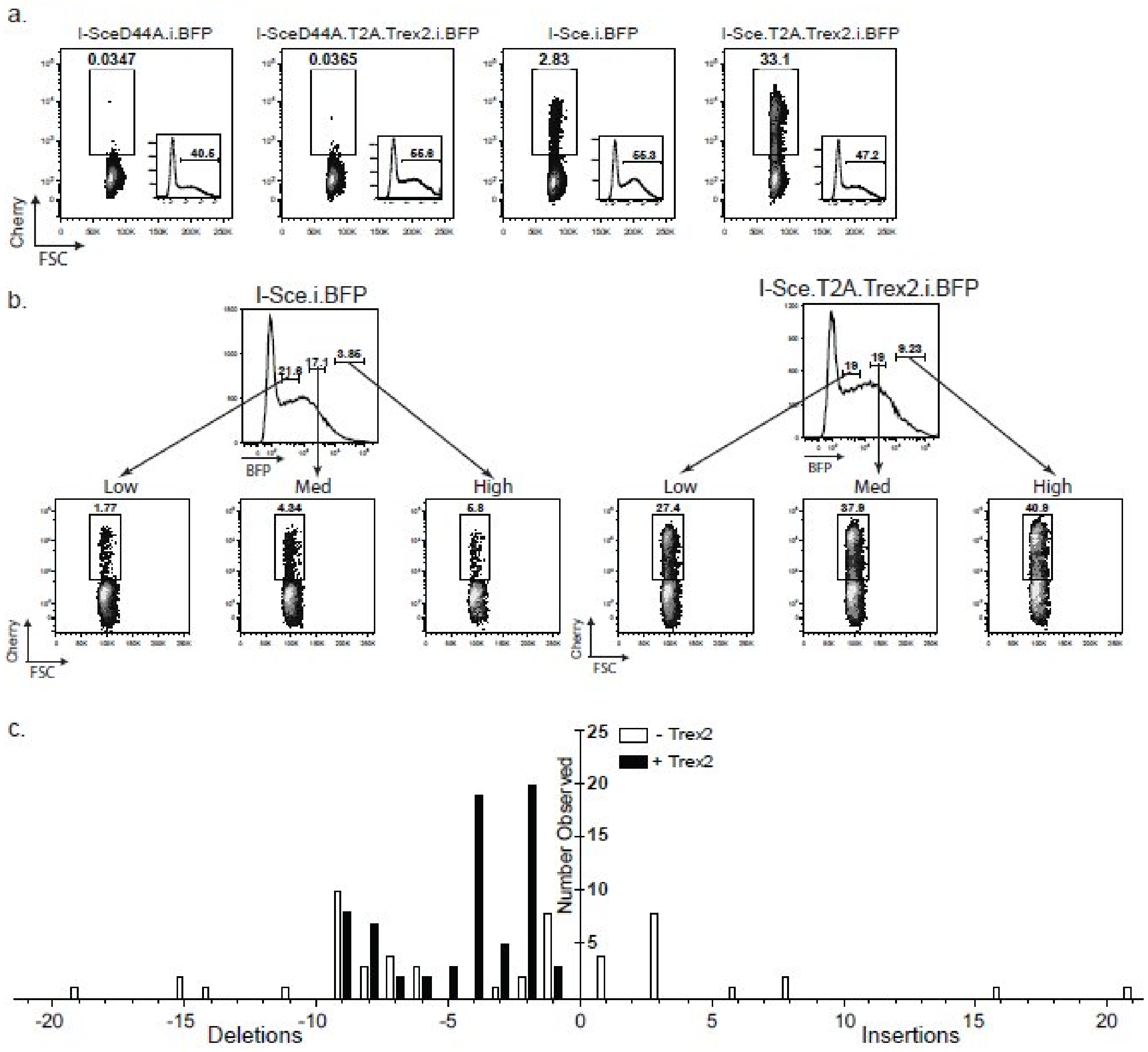
Supplementary Figure 1. Schematic of the Traffic Light Reporter (TLR).

(**a**). Schematic of the TLR locus harbouring the I-SceI target site embedded within the +1 GFP reading frame, followed by the T2A.mCherry ORFs in the +3 reading frame. (**b**). Diagram of the TLR outcomes following repair pathway choice. A fluorescent GFP protein will be translated if a homology-direct repair (HDR) event occurs, while a fluorescent mCherry protein will be expressed if a mutagenic NHEJ event occurs that results in a +3 frameshift.

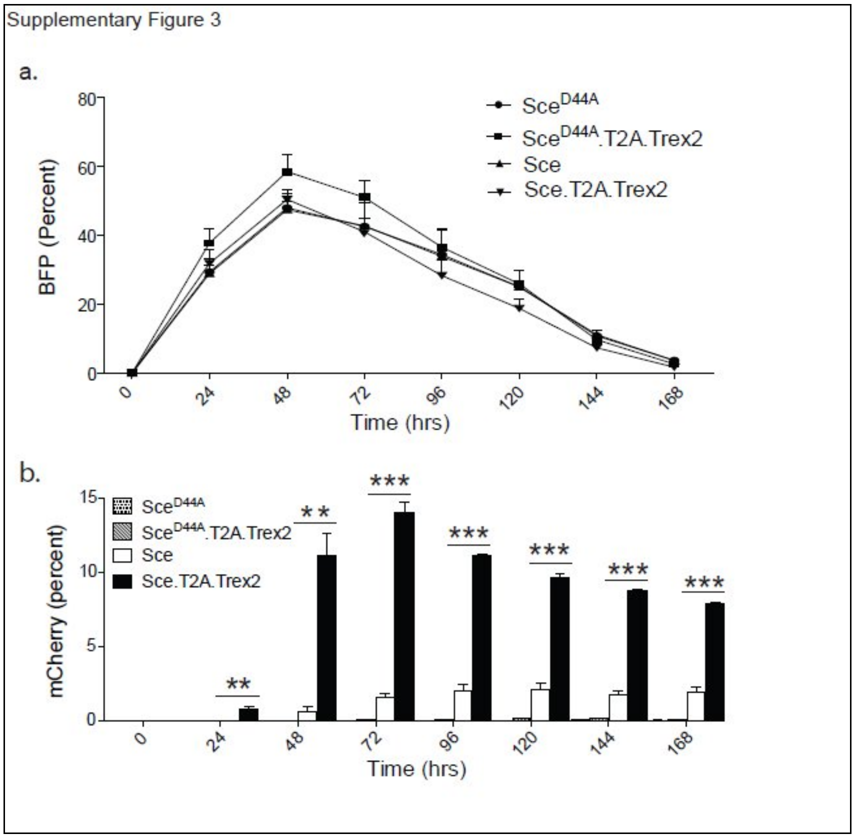


Supplementary Figure 2. Trex2 increases gene disruption frequency of I-SceI mediated breaks (**a**). Representative flow plots 72 hours post transfection of HEK293T traffic light reporter cells with indicated vectors. Cherry indicates gene disruption rates observed in transfected cells, inset flow plots show gating for BFP positive cells to normalize for transfection efficiency. (**b**) Gating analysis of BFP fluorescence (analyzed as low, med, and high) of HEK293T TLR-Sce cells following transfection with the indicated vectors. (**c**) Sizes of insertions and deletions found in HEK293T TLR-Sce cells with or without Trex2.

Supplementary Figure 2

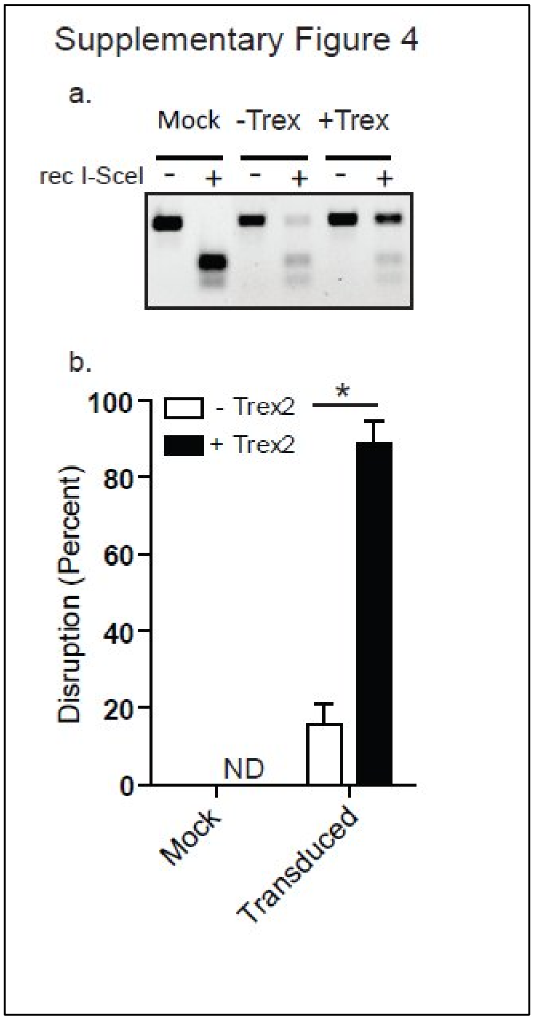


Supplementary Figure 3. Kinetic analysis of repair.
(**a**). Analysis of BFP expression at the time-points indicated following transfection with the indicated vectors. The constructs shown are tagged to BFP by an IRES sequence downstream of either I-SceI or Trex2. (**b**) Quantification of 3 experiments of HEK293T cells transfected with the indicated vectors, analyzed at the time-points indicated. Cherry indicates gene disruption rates observed in transfected cells.



Supplementary Figure 4. Trex2 increases the gene disruption effiency of I-SceI in primary MEFs.

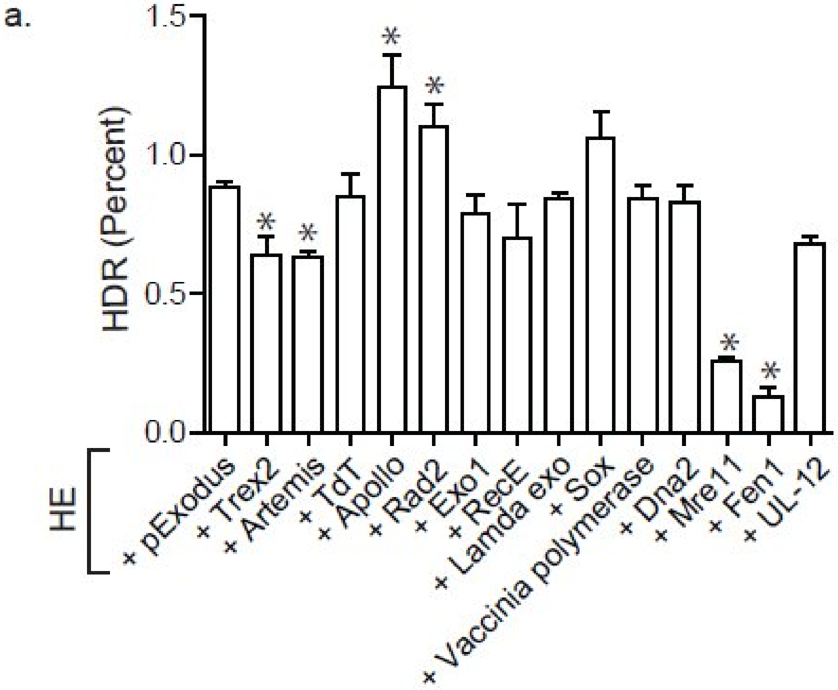
(**a**) Digestion of PCR amplified I-SceI site in primary MEFs derived from the “Sce-SCID” mouse model 72hrs post transduction with Sce.i.BFP and Sce.T2A.Trex2.i.BFP LV at an MOI of 10. Recombinant (rec) I-SceI resistant bands indicate gene disruption. (**b**) Quantitation of 2 independent experiments as performed in a. ND, not determined.



Supplementary Figure 5. Rates of homology-directed repair (HDR) in cells expressing I-SceI and an end-processing enzyme.

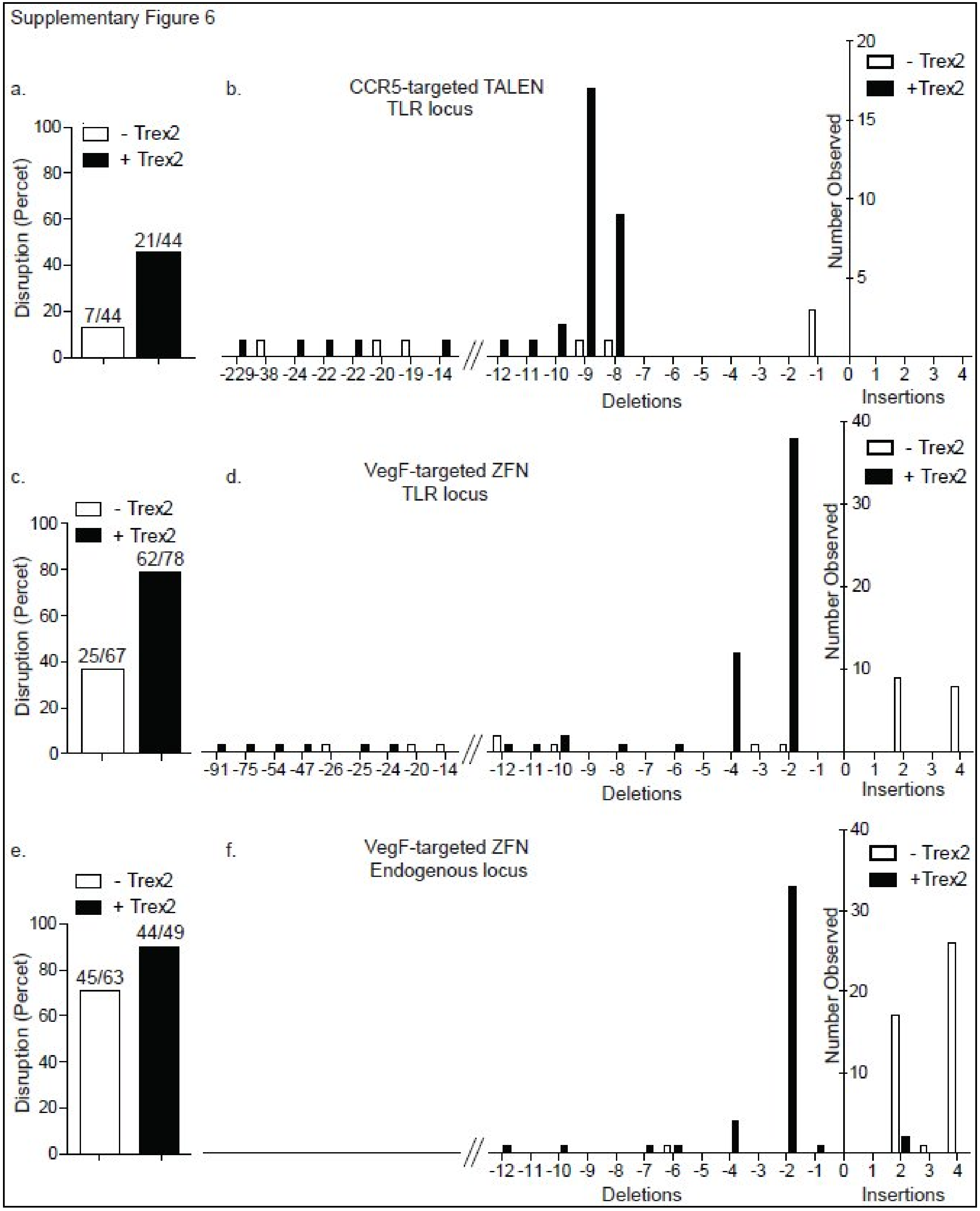
(**a**). Quantitation of homology-directed repair (HDR) of 3 independent experiments of HEK293T I-SceI TLR cells expressing I-SceI and either pExodus (control empty vector) or an end-processing enzyme. Measurement of HDR is based on GFP fluorescence from the Traffic Light reporter assay, 72 hours post-transfection. Error bars represent standard error of the mean (SEM), and *p*-values (with * representing *p*<0.05) were calculated using the Student's two-tailed unpaired t-test. *p* values indicate a significant difference between samples expressing an enzyme from the library with designer endonuclease, compared to endonuclease with pExodus.

Supplementary Figure 5

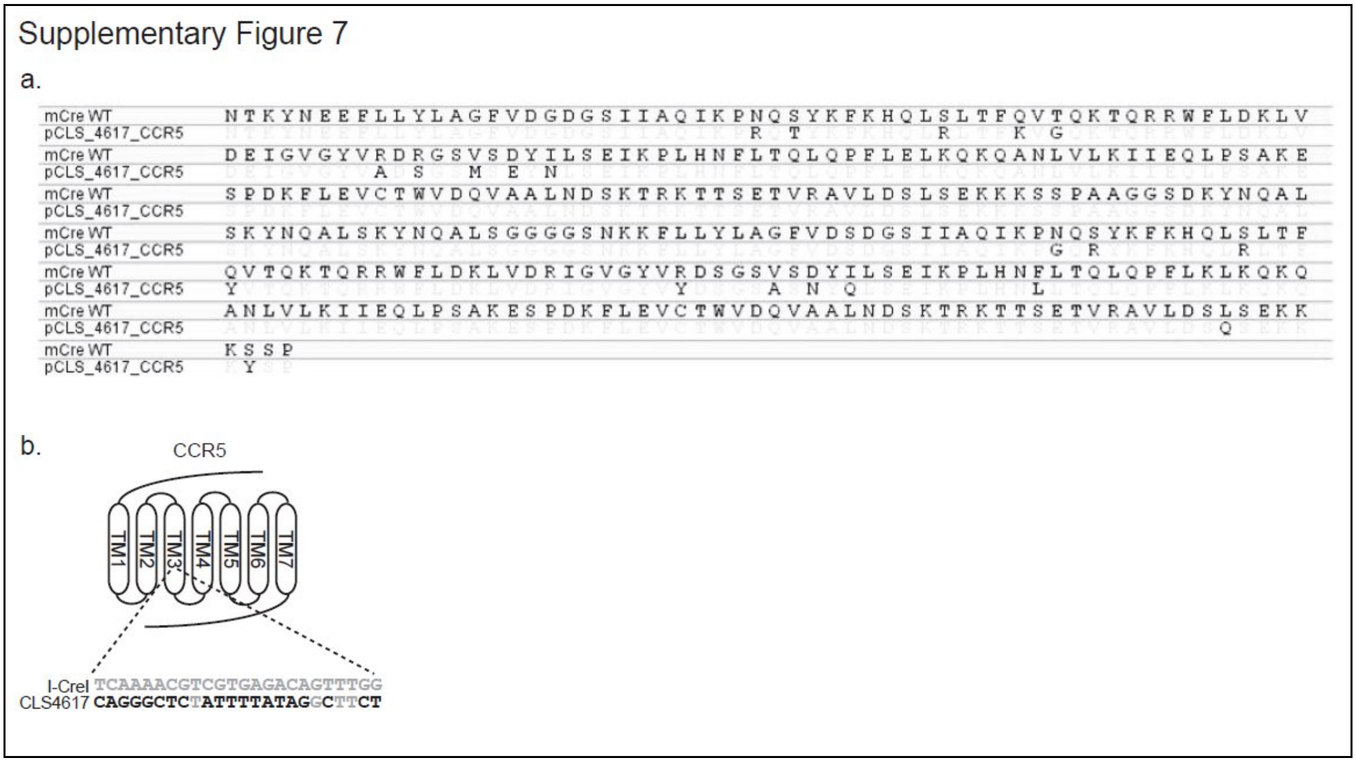


Supplementary Figure 6. Modification rates and mutation spectra of TALEN or ZFN with and without Trex2 by sequencing analysis.

(**a, c, e**). Quantification of overall disruption rates by CCR5 TALEN at TLR locus (a), VegF ZFN at TLR locus (c) or endogenous locus (e) with or without Trex2. Numbers above bars represent mutated sequences over total sequences analyzed. (**b, d, f**). Base pair modifications induced by CCR5 TALEN (b), or VegF ZFN (d, f) with or without Trex2.

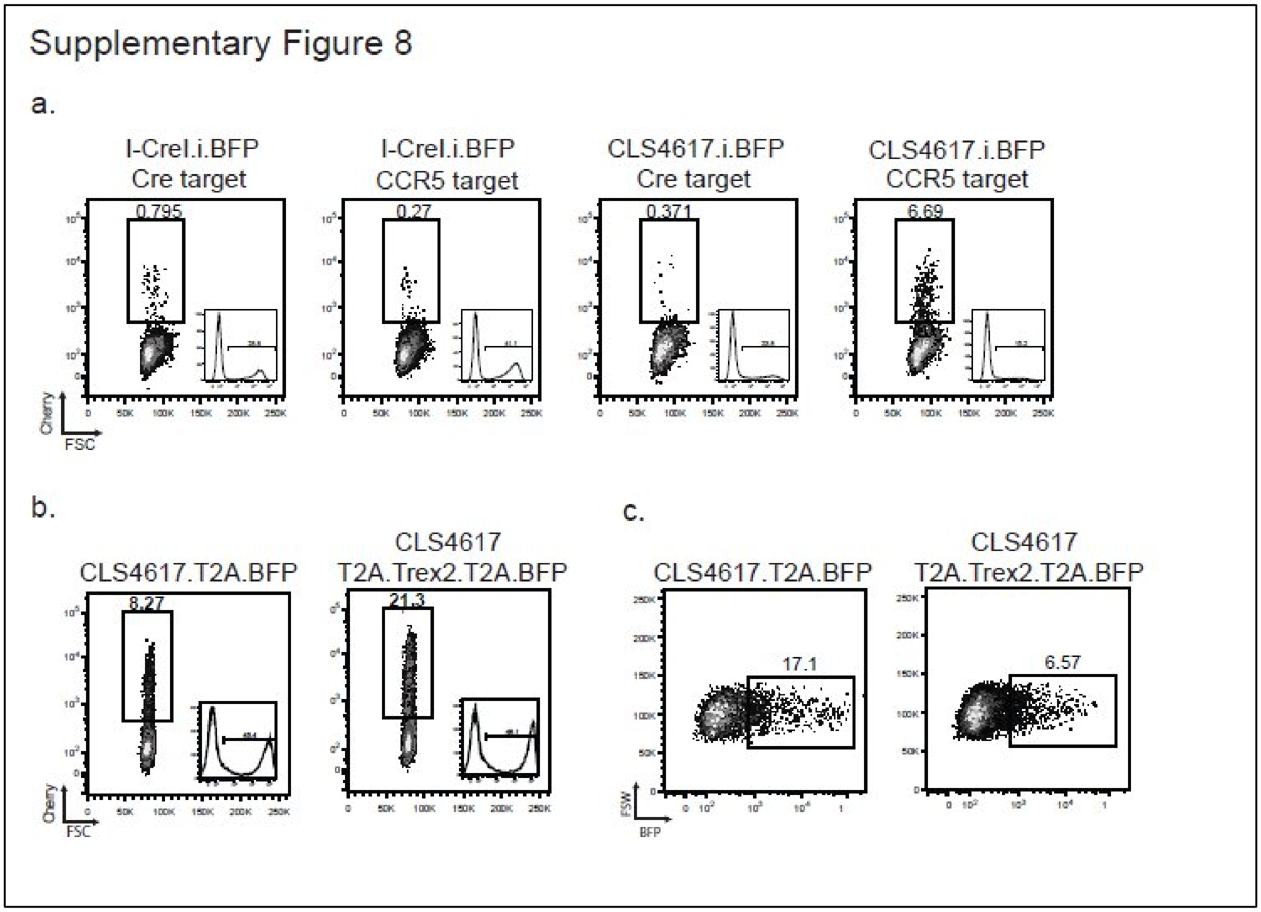


Supplementary Figure 7. Sequence alignment and schematic CLS4617 with mCreWT. (**a**). mCreWT refers to the sequence of the wild-type monomerized Cre enzyme, while pCLS_4617_CCR5 refers to the amino acid residues changed in the re-designed enzyme, referred to as CLS4617. (**b**). Schematic of the CLS4617 target site, compared to the I-CreI target site, within the third transmembrane domain of the CCR5 gene.

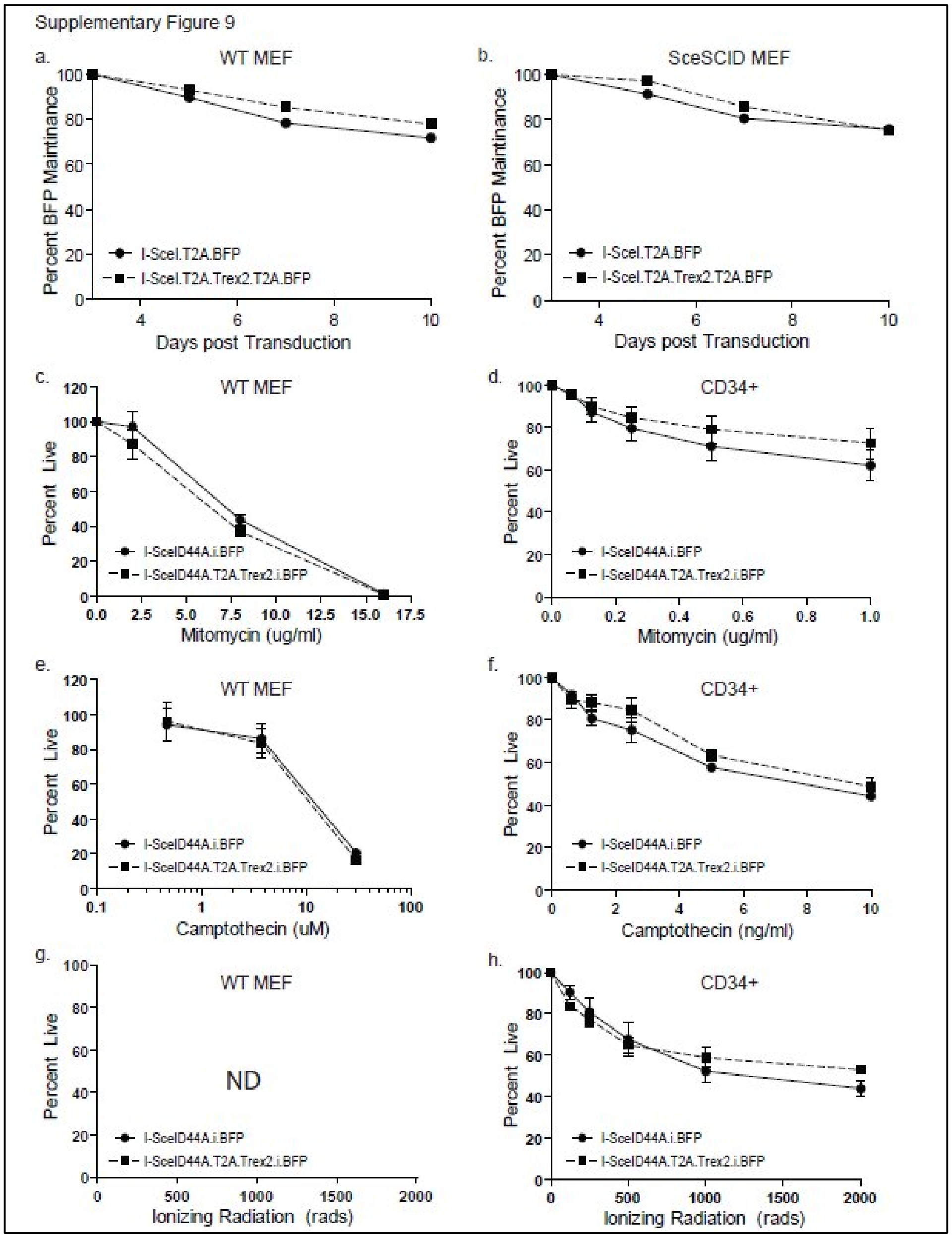


Supplementary Figure 8. Trex2 increases knockout of CCR5 with an engineered homing endonuclease.

(**a**) Representative flow plots from HEK293T cells containing traffic light reporters with the I-CreI cognate and CLS4617 targets following transfection with each enzyme. (**b**) Representative flow plot following transfection of HEK293T traffic light reporter cells with CLS4617 target with the indicated vectors. (**c**) Representative flow plot following transduction of CD34$^+$ cells with lentiviral vectors at an MOI of 20.



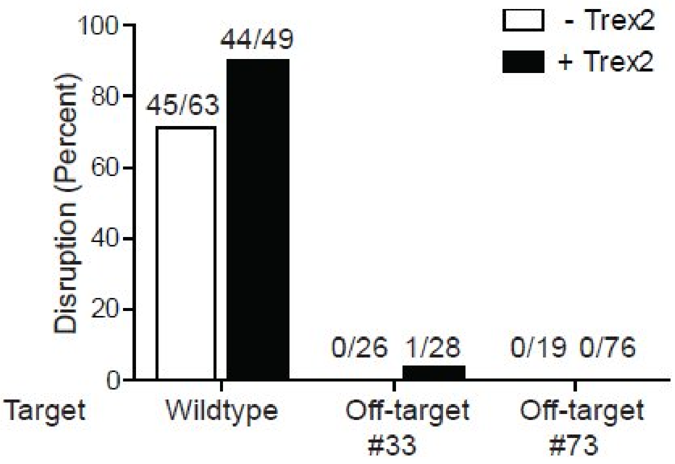
Supplementary Figure 9. Trex2 does not cause toxicity or sensitivity to DNA damaging agents. (**a,b**). BFP maintenance in WT MEF (a) and SceSCID MEF (b) primary cells expressing I-SceI with or without co-expression of Trex2 over 10 day period. (**c, d**). Survival of WT MEFs (c) or CD34+ human primary cells (d) by quantification of propidium iodide negative cells following transduction with indicated recombinant LV, and treatment with mitomycin C at the indicated doses. (**e, f**) Survival of WT MEFs (e) or CD34+ human primary cells (f) by quantification of propidium iodide negative cells following transduction with indicated recombinant LV, and treatment with camptothecin at the indicated doses. (**g**). Survival of WT MEFs transduced with the indicated vectors and treated with ionizing radiation was not determined (ND), as no death was detectable with the indicated doses of radiation. (**h**) Survival of CD34+ human primary cells following transduction with indicated recombinant LV, and treatment with ionizing radiation at the indicated doses.



Supplementary Figure 10. Off-target effects of VegF ZFN with or without Trex2.
(**a**). Sequence analysis of mutations induced by VegF ZFN with or without Trex2 at the indicated genomic loci. Off-targets refer to published loci[17]. Numbers above bars represent mutated sequences over total sequences analyzed.

Supplementary Figure 10

a.



Supplementary Tables

Supplementary Table 1: Homing endonucleases used in this study and their target sites.

Homing Endonucleases	Target
I-SceI	TAGGGATAACAGGGTAAT
I-LtrI	AATGCTCCTATACGACGTTTAG
I-GpiI	TTTTCCTGTATATGACTTAAAT
I-GzeI	GCCCCTCATAACCCGTATCAAG
I-xMpeMI (unpublished, A.R.L., A.M.S.)	TAGATAACCATAAGTGGCTAAT
I-PanMI	GCTCCTCATAATCCTTATCAAG
I-CreI	TCAAAACGTCGTGAGACAGTTTGG
I-OnuI	TTTCCACTTATTCAACCTTTTA
I-HjeMI	TTGAGGAGGTTTCTCTGTTAAT
I-AniI	TGAGGAGGTTTCTCTGTAAA
CLS4617	CAGGGCTCTATTTTATAGGCTTCT

Supplementary Table 2: Library of DNA end-processing enzymes.

Enzyme	**Gene name**	**Activity**	**Species of origin**	**NLS added**	**Reference**
Apollo	SNM1B	5-3’ exonuclease	Human	No	Lenain *et al*[1]
Artemis	Artemis	5-3’ exonuclease	Human	No	Kurosawa *et al* [2]
Dna2	DNA2	5-3’ exonuclease, helicase	Human	No	Nimonkar *et al* [3]
Exo1	EXO1	5-3’ exonuclease	Human	No	Nimonkar *et al* [3], Orans *et al* [4]
Fen1	FEN1	5’ flap endonuclease	Human	No	Jagannathan *et al* [5], Tsutakawa *et al* [6]
Mre11	MRE11	5-3’ and 3-5’ exonuclease	Human	No	Garcia *et al* [7]
Rad2	n/a (catalytic domain of Exo1)	5-3’ exonuclease (Exo1 catalytic domain)	Human	No	Lee and Wilson [8]
TdT (terminal deoxynucleotidyl transferase)	TdT	Single-stranded template-independent DNA polymerase	Human	No	Mahajan *et al* [9]
RecE	RecE	5-3’ exonuclease	*E. coli*	Yes	Zhang *et al* [10]
Lambda exonuclease	λ exonuclease	5-3’ exonuclease	Bacteriophage λ	Yes	Zhang *et al* [11]
Sox (T24I mutation)	SOX	5-3’ alkaline exonuclease	Kaposi’s sarcoma-associated herpesvirus	Yes	Glaunsinger *et al*[12]; Daltroth *et al*[13]
Vaccinia DNA polymerase	E9L	3-5’ exonuclease	Vaccinia poxvirus	Yes	Gammon and Evans[14]
UL-12	UL12	5-3’ alkaline exonuclease	Herpes simplex virus (HSV)-1	Yes	Reuven *et al*[15]; Balasubramanian *et al*[16]

Supplemental References

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15. Reuven, N.B., Staire, A.E., Myers, R.S. & Weller, S.K. The herpes simplex virus type 1 alkaline nuclease and single-stranded DNA binding protein mediate strand exchange in vitro. *J Virol* 77, 7425-7433 (2003).
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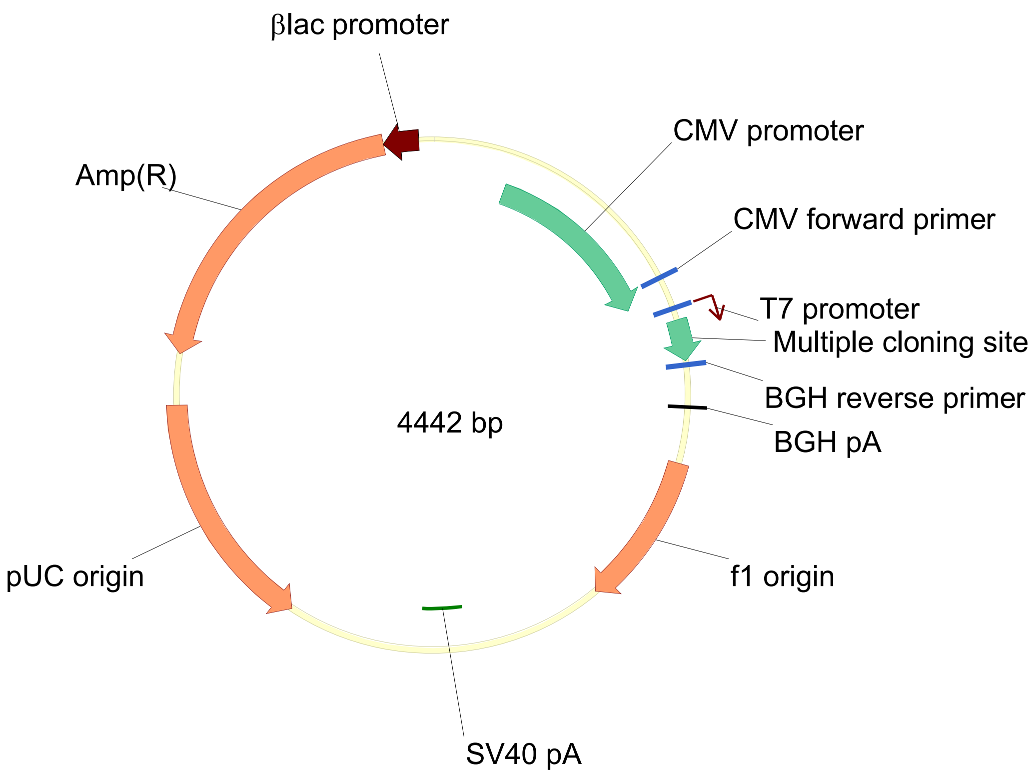
17. Pattanayak, V., Ramirez, C.L., Joung, J.K., Liu, D.R. Revealing off-target cleavage specifticties of zinc-finger nucleases by *in vitro* selection. *Nat Meth* 8, 765-770 (2011).

Supplementary Note 1: Vector Maps

pExodus



General Description

DNA Exodus MCS

Entire molecule length: 4442 bp

Feature Map

CDS (1 total)

Amp(R)

Start: 3446 End: 4306 (Complementary)

Original Location Description:

complement(3446..4306)

Qualifiers:

/invitrogen="1020000"

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  - MCS
    - Start: 895   End: 1010
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      - 895..1010
    - Qualifiers:
      - /invitrogen="1630000"
- PolyA Signal (1 total)
  - SV40 pA
    - Start: 2118 End: 2248
    - Original Location Description:
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    - Qualifiers:
      - /invitrogen="1840000"
- PolyA Site (1 total)
  - BGH pA
    - Start: 1028 End: 1252
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      - 1028..1252
    - Qualifiers:
      - /invitrogen="1870000"
- Promoter Eukaryotic (1 total)
  - CMV promoter
    - Start: 232   End: 819
    - Original Location Description:
      - 232..819
    - Qualifiers:
      - /invitrogen="2180000"
- Promoter Prokaryotic (2 total)
  - T7 promoter
    - Start: 863   End: 879

Original Location Description:

863..879

Qualifiers:

/invitrogen="2370000"

bla promoter

Start: 4307 End: 4405 (Complementary)

Original Location Description:

complement(4307..4405)

Qualifiers:

/invitrogen="2330000"

Replication Origin (2 total)

f1 origin

Start: 1298 End: 1726

Original Location Description:

1298..1726

Qualifiers:

/invitrogen="2500000"

pUC origin

Start: 2631 End: 3301 (Complementary)

Original Location Description:

complement(2631..3301)

Qualifiers:

/invitrogen="2540000"

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301 tggagttccg cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt gacgtcaata atgacgtatg ttcccatagt

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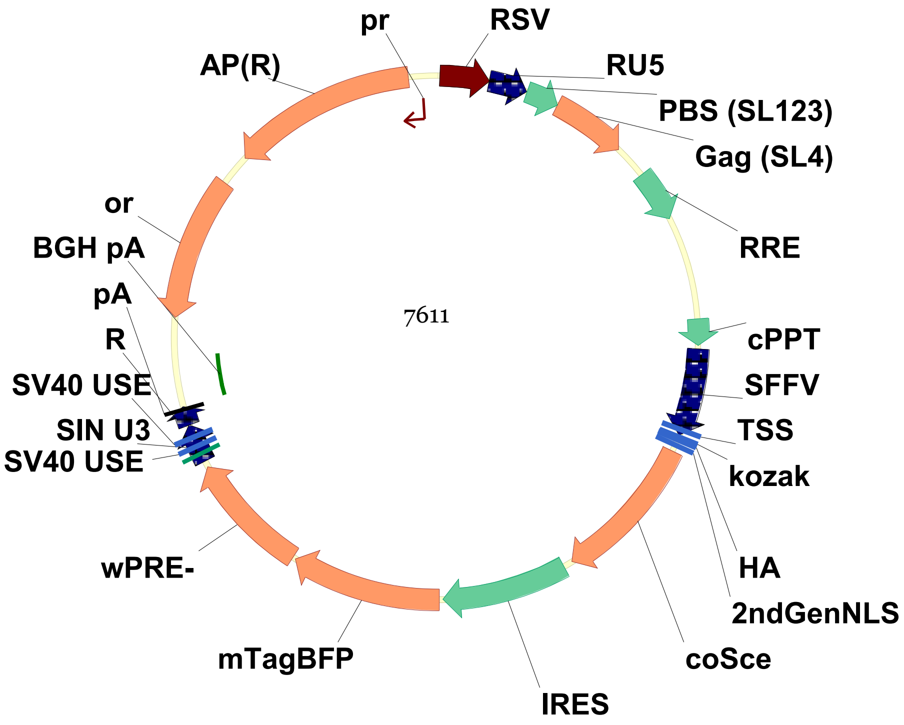
pCVL.SFFV.HA.NLS.Sce(Opt).IRES.mTagBFP



General Description

DNA Plasmid pCVL.SFFV.HA.NLS.SceOpt.IRES.mTagBFP

Entire molecule length: 7611 bp

Feature Map

- CDS (5 total)
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    - Original Location Description:
      - 572..924
  - coSce
    - Start: 2438 End: 3139
    - Original Location Description:
      - 2438..3139
  - mTagBFP
    - Start: 3784 End: 4485
    - Original Location Description:
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  - wPRE-O
    - Start: 4500 End: 5080
    - Original Location Description:
      - 4500..5080
  - AP(R)
    - Start: 6621 End: 7481 (Complementary)
    - bla gene- Ap(r) determinant
    - Original Location Description:
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- LTR (4 total)
  - RU5
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  - SFFV

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PBS (SL123)
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RRE
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    1081..1322
cPPT
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    1806..1929
kozak
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  Original Location Description:
    2366..2374
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ATG

Start: 2375 End: 2377

Original Location Description:

2375..2377

HA

Start: 2381 End: 2407

Original Location Description:

2381..2407

2ndGenNLS

Start: 2408 End: 2431

Original Location Description:

2408..2431

IRES

Start: 3183 End: 3768

Original Location Description:

3183..3768

SV40 USE

Start: 5186 End: 5229

Original Location Description:

5186..5229

SV40 USE

Start: 5233 End: 5276

Original Location Description:

5233..5276

PolyA Signal (1 total)

BGH pA

Start: 5385 End: 5618

Original Location Description:

5385..5618

PolyA Site (1 total)

pA

Start: 5367 End: 5372

Original Location Description:

5367..5372

Primer (1 total)

Promoter Prokaryotic (2 total)

RSV

Start: 7 End: 235

Original Location Description:

7..235

pr

Start: 7546 End: 7551 (Complementary)

Original Location Description:

complement(7546..7551)

Replication Origin (1 total)

ori

Start: 5800 End: 6473 (Complementary)

Original Location Description:

complement(5800..6473)

mRNA (1 total)

TSS

Start: 2330 End: 2355

Original Location Description:

2330..2355

Sequence

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101 cgattggtgg aagtaaggtg gtacgatcgt gccttattag gaaggcaaca gacgggtctg acatggattg gacgaaccac tgaattgccg cattgcagag

201 atattgtatt taagtgccta gctcgataca taaacgggtc tctctggtta gaccagatct gagcctggga gctctctggc taactaggga acccactgct

301 taagcctcaa taaagcttgc cttgagtgct tcaagtagtg tgtgcccgtc tgttgtgtga ctctggtaac tagagatccc tcagaccctt ttagtcagtg

401 tggaaaatct ctagcagtgg cgcccgaaca gggacttgaa agcgaaaggg aaaccagagg agctctctcg acgcaggact cggcttgctg aagcgcgcac

501 ggcaagaggc gaggggcggc gactggtgag tacgccaaaa attttgacta gcggaggcta gaaggagaga gatgggtgcg agagcgtcag tattaagcgg

601 gggagaatta gatcgcgatg ggaaaaaatt cggttaaggc cagggggaaa gaaaaaatat aaattaaaac atatagtatg ggcaagcagg gagctagaac

701 gattcgcagt taatcctggc ctgttagaaa catcagaagg ctgtagacaa atactgggac agctacaacc atcccttcag acaggatcag aagaacttag

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901 aaaagtaaga ccaccgcaca gcaagcggcc ctgatcttca gacctggagg aggagatatg agggacaatt ggagaagtga attatataaa tataaagtag

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1801 cggttaactt ttaaaagaaa aggggggatt ggggggtaca gtgcagggga aagaatagta gacataatag caacagacat acaaactaaa gaattacaaa

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2001 agaagttcag atcaagggcg ggtacatgaa aatagctaac gttgggccaa acaggatatc tgcggtgagc agtttcggcc ccggcccggg gccaagaaca

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2401 ttatgcgcca cctaagaaga aacgcaaagt cgaattcaag aacatcaaga agaaccaggt catgaacctg ggccccaaca gcaagctgct gaaggagtac

2501 aagagccagc tgatcgagct gaacatcgag cagttcgagg ccggcatcgg cctgatcctg ggcgacgcct acatcaggag cagggacgag ggcaagacct

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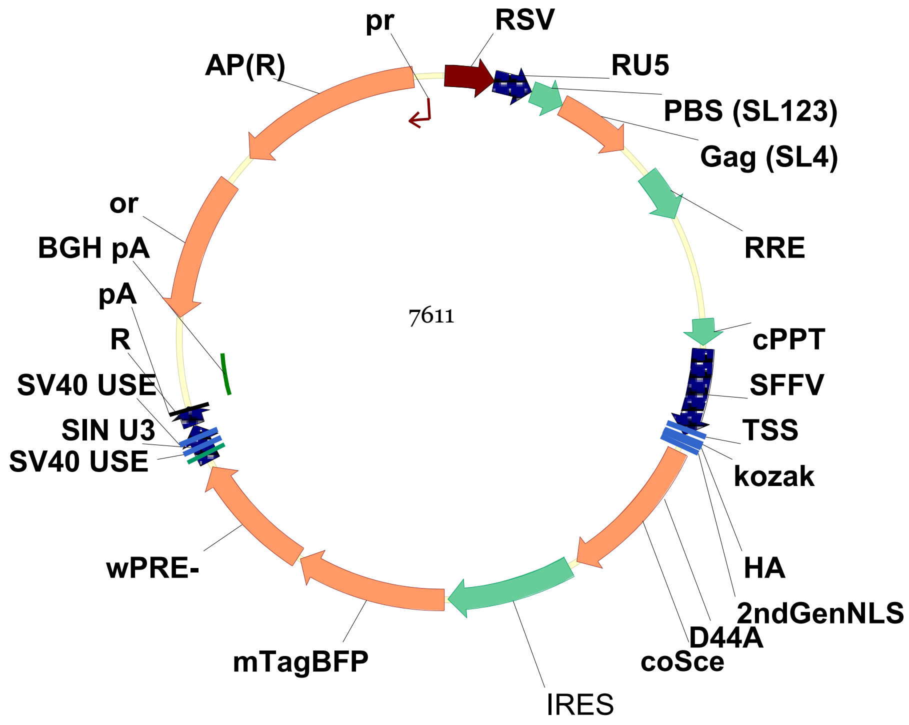

General Description

DNA Plasmid pCVL.SFFV.HA.NLS.SceOptD44A.IRES.mTagBFP

Entire molecule length: 7611 bp

Feature Map

CDS (5 total)

Gag (SL4)

Start: 572 End: 924

Original Location Description:

572..924

coSce

Start: 2438 End: 3139

Original Location Description:

2438..3139

mTagBFP

Start: 3784 End: 4485

Original Location Description:

3784..4485

wPRE-O

Start: 4500 End: 5080

Original Location Description:

4500..5080

AP(R)

Start: 6621 End: 7481 (Complementary)

bla gene- Ap(r) determinant

Original Location Description:

complement(6621..7481)

LTR (4 total)

RU5

Start: 236 End: 417

Original Location Description:

236..417

SFFV

Start: 1946 End: 2355

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    1946..2355
  Qualifiers:
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    /product="#AJ224005"
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SIN U3
  Start: 5112  End: 5293
  Original Location Description:
    5112..5293
R
  Start: 5294  End: 5383
  Original Location Description:
    5294..5383
PBS (SL123)
  Start: 418   End: 571
  Original Location Description:
    418..571
RRE
  Start: 1081  End: 1322
  Original Location Description:
    1081..1322
cPPT
  Start: 1806  End: 1929
  Original Location Description:
    1806..1929
kozak
  Start: 2366  End: 2374
  Original Location Description:
    2366..2374
ATG
```

Start: 2375 End: 2377

Original Location Description:

2375..2377

HA

Start: 2381 End: 2407

Original Location Description:

2381..2407

2ndGenNLS

Start: 2408 End: 2431

Original Location Description:

2408..2431

IRES

Start: 3183 End: 3768

Original Location Description:

3183..3768

SV40 USE

Start: 5186 End: 5229

Original Location Description:

5186..5229

SV40 USE

Start: 5233 End: 5276

Original Location Description:

5233..5276

PolyA Signal (1 total)

BGH pA

Start: 5385 End: 5618

Original Location Description:

5385..5618

PolyA Site (1 total)

pA

Start: 5367 End: 5372

Original Location Description:

5367..5372

Primer (1 total)

Promoter Prokaryotic (2 total)

RSV

Start: 7 End: 235

Original Location Description:

7..235

pr

Start: 7546 End: 7551 (Complementary)

Original Location Description:

complement(7546..7551)

Replication Origin (1 total)

ori

Start: 5800 End: 6473 (Complementary)

Original Location Description:

complement(5800..6473)

mRNA (1 total)

TSS

Start: 2330 End: 2355

Original Location Description:

2330..2355

Sequence

```
  1 gacgtcaatg tagtcttatg caatactctt gtagtcttgc aacatggtaa cgatgagtta gcaacatgcc ttacaaggag agaaaaagca ccgtgcatgc

101 cgattggtgg aagtaaggtg gtacgatcgt gccttattag gaaggcaaca gacgggtctg acatggattg gacgaaccac tgaattgccg cattgcagag

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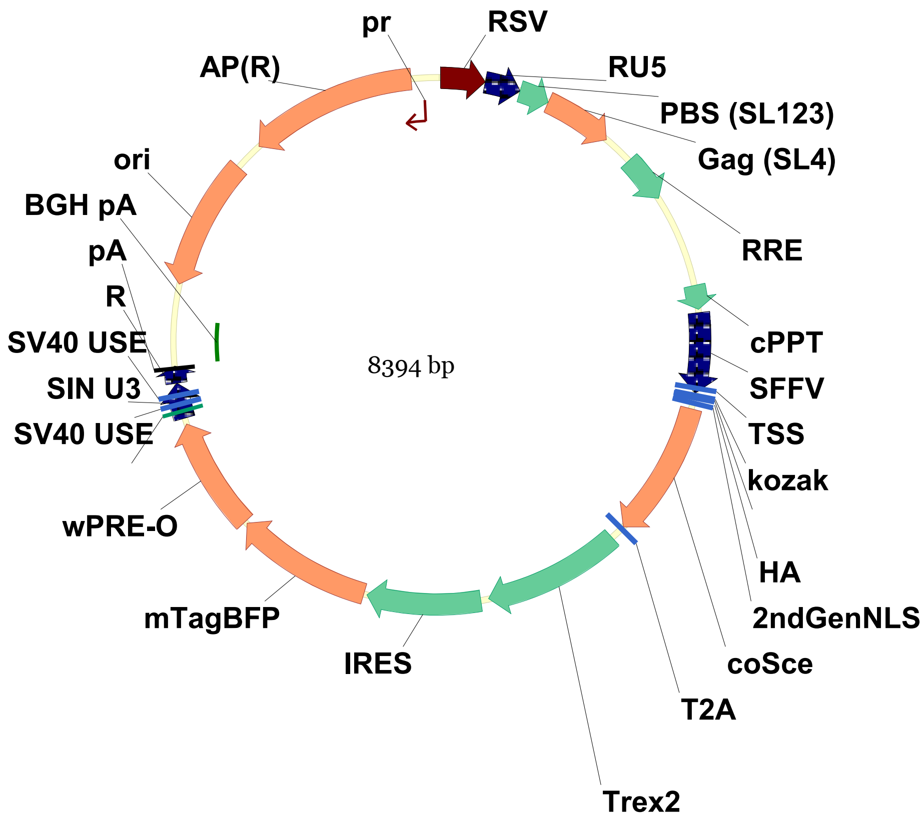

General Description

DNA Plasmid pCVL.SFFV.HA.NLS.SceOpt.T2A.Trex2.IRES.mTagBFP

Entire molecule length: 8394 bp

Feature Map

CDS (5 total)

Gag (SL4)

Start: 572 End: 924

Original Location Description:

572..924

coSce

Start: 2438 End: 3139

Original Location Description:

2438..3139

mTagBFP

Start: 4567 End: 5268

Original Location Description:

4567..5268

wPRE-O

Start: 5283 End: 5863

Original Location Description:

5283..5863

AP(R)

Start: 7404 End: 8264 (Complementary)

bla gene- Ap(r) determinant

Original Location Description:

complement(7404..8264)

LTR (4 total)

RU5

Start: 236 End: 417

Original Location Description:

236..417

SFFV

Start: 1946 End: 2355

Original Location Description:

1946..2355

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 SIN U3
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  Original Location Description:
    5895..6076
 R
  Start: 6077  End: 6166
  Original Location Description:
    6077..6166
Misc. Feature (11 total)
 PBS (SL123)
  Start: 418   End: 571
  Original Location Description:
    418..571
 RRE
  Start: 1081  End: 1322
  Original Location Description:
    1081..1322
 cPPT
  Start: 1806  End: 1929
  Original Location Description:
    1806..1929
 kozak
  Start: 2366  End: 2374
  Original Location Description:
    2366..2374
 ATG
  Start: 2375  End: 2377
```

Original Location Description:

2375..2377

HA

Start: 2381 End: 2407

Original Location Description:

2381..2407

2ndGenNLS

Start: 2408 End: 2431

Original Location Description:

2408..2431

Trex2

Start: 3218 End: 3925

Original Location Description:

3218..3925

IRES

Start: 3966 End: 4551

Original Location Description:

3966..4551

PolyA Signal (1 total)

BGH pA

Start: 6168 End: 6401

Original Location Description:

6168..6401

PolyA Site (1 total)

pA

Start: 6150 End: 6155

Original Location Description:

6150..6155

Primer (1 total)

CVL-34

Start: 1671 End: 1692

Original Location Description:

1671..1692

Promoter Prokaryotic (2 total)

RSV

Start: 7 End: 235

Original Location Description:

7..235

pr

Start: 8329 End: 8334 (Complementary)

Original Location Description:

complement(8329..8334)

Replication Origin (1 total)

ori

Start: 6583 End: 7256 (Complementary)

Original Location Description:

complement(6583..7256)

mRNA (1 total)

TSS

Start: 2330 End: 2355

Original Location Description:

2330..2355

Sequence

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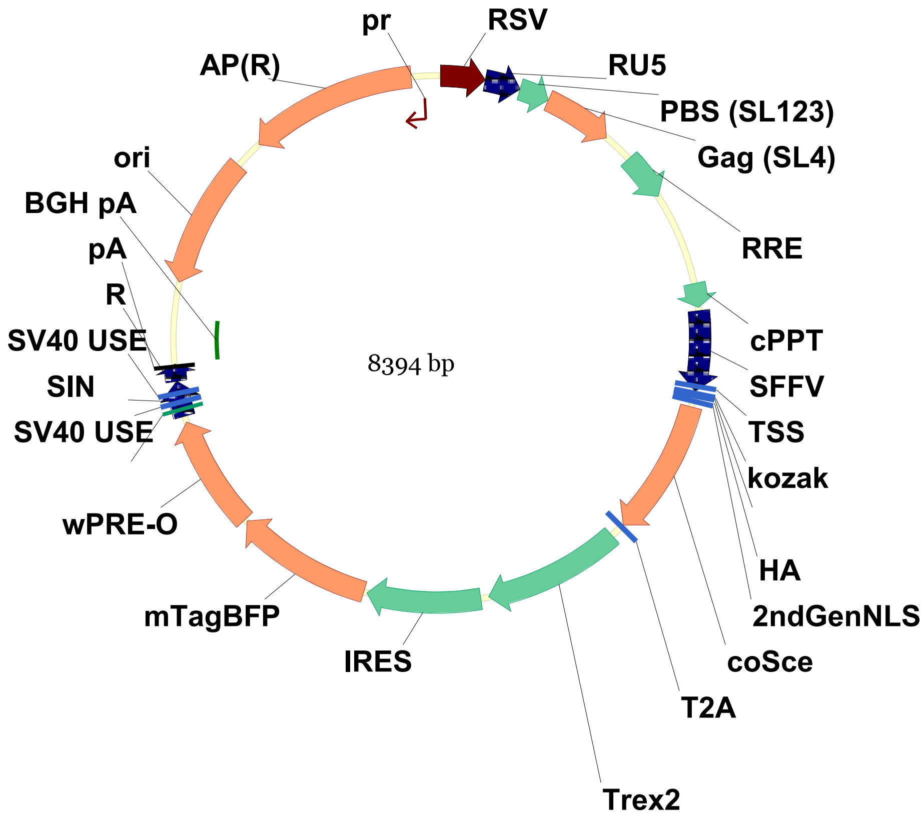

General Description

DNA Plasmid pCVL.SFFV.HA.NLS.SceOptD44A.T2A.Trex2.IRES.mTagBFP

Entire molecule length: 8394 bp

Feature Map

CDS (5 total)

Gag (SL4)

Start: 572 End: 924

Original Location Description:

572..924

coSce

Start: 2438 End: 3139

Original Location Description:

```
      2438..3139
  mTagBFP
    Start: 4567  End: 5268
    Original Location Description:
      4567..5268
  wPRE-O
    Start: 5283  End: 5863
    Original Location Description:
      5283..5863
  AP(R)
    Start: 7404  End: 8264 (Complementary)
    bla gene- Ap(r) determinant
    Original Location Description:
      complement(7404..8264)
LTR (4 total)
  RU5
    Start: 236   End: 417
    Original Location Description:
      236..417
  SFFV
    Start: 1946  End: 2355
    Original Location Description:
      1946..2355
    Qualifiers:
      /gene="'MESV"
      /product="#AJ224005"
      /SECDrawAs="Gene"
  SIN U3
    Start: 5895  End: 6076
    Original Location Description:
      5895..6076
```

R

Start: 6077 End: 6166

Original Location Description:

6077..6166

Misc. Feature (11 total)

PBS (SL123)

Start: 418   End: 571

Original Location Description:

418..571

RRE

Start: 1081 End: 1322

Original Location Description:

1081..1322

cPPT

Start: 1806 End: 1929

Original Location Description:

1806..1929

kozak

Start: 2366 End: 2374

Original Location Description:

2366..2374

ATG

Start: 2375 End: 2377

Original Location Description:

2375..2377

HA

Start: 2381 End: 2407

Original Location Description:

2381..2407

2ndGenNLS

Start: 2408 End: 2431

Original Location Description:

2408..2431

Trex2

Start: 3218 End: 3925

Original Location Description:

3218..3925

IRES

Start: 3966 End: 4551

Original Location Description:

3966..4551

PolyA Signal (1 total)

BGH pA

Start: 6168 End: 6401

Original Location Description:

6168..6401

PolyA Site (1 total)

pA

Start: 6150 End: 6155

Original Location Description:

6150..6155

Primer (1 total)

CVL-34

Start: 1671 End: 1692

Original Location Description:

1671..1692

Promoter Prokaryotic (2 total)

RSV

Start: 7 End: 235

Original Location Description:

7..235

pr

Start: 8329 End: 8334 (Complementary)

Original Location Description:

complement(8329..8334)

Replication Origin (1 total)

ori

Start: 6583 End: 7256 (Complementary)

Original Location Description:

complement(6583..7256)

mRNA (1 total)

TSS

Start: 2330 End: 2355

Original Location Description:

2330..2355

Sequence

1 gacgtcaatg tagtcttatg caatactctt gtagtcttgc aacatggtaa cgatgagtta gcaacatgcc ttacaaggag agaaaaagca ccgtgcatgc

101 cgattggtgg aagtaaggtg gtacgatcgt gccttattag gaaggcaaca gacgggtctg acatggattg gacgaaccac tgaattgccgcattgcagag

201 atattgtatt taagtgccta gctcgataca taaacgggtc tctctggtta gaccagatct gagcctggga gctctctggc taactaggga acccactgct

301 taagcctcaa taaagcttgc cttgagtgct tcaagtagtg tgtgcccgtc tgttgtgtga ctctggtaac tagagatccc tcagaccctt ttagtcagtg

401 tggaaaatct ctagcagtgg cgcccgaaca gggacttgaa agcgaaaggg aaaccagagg agctctctcg acgcaggact cggcttgctgaagcgcgcac

501 ggcaagaggc gaggggcggc gactggtgag tacgccaaaa attttgacta gcggaggcta gaaggagaga gatgggtgcg agagcgtcag tattaagcgg

601 gggagaatta gatcgcgatg ggaaaaaatt cggttaaggc cagggggaaa gaaaaaatat aaattaaaac atatagtatg ggcaagcagg gagctagaac

701 gattcgcagt taatcctggc ctgttagaaa catcagaagg ctgtagacaa atactgggac agctacaacc atcccttcag acaggatcag aagaacttag

801 atcattatat aatacagtag caaccctcta ttgtgtgcat caaaggatag agataaaaga caccaaggaa gctttagaca agatagagga agagcaaaac

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1401 ttggaatcac acgacctgga tggagtggga cagagaaatt aacaattaca caagcttaat acactcctta attgaagaat cgcaaaacca gcaagaaaag

1501 aatgaacaag aattattgga attagataaa tgggcaagtt tgtggaattg gtttaacata acaaattggc tgtggtatat aaaattattc ataatgatag

1601 taggaggctt ggtaggttta agaatagttt ttgctgtact ttctatagtg aatagagtta ggcagggata ttcaccatta tcgtttcaga cccacctccc

1701 aaccccgagg ggacccgaca ggcccgaagg aatagaagaa gaaggtggag agagagacag agacagatcc attcgattag tgaacggatc tcgacggtat

1801 cggttaactt ttaaaagaaa aggggggatt ggggggtaca gtgcagggga aagaatagta gacataatag caacagacat acaaactaaa gaattacaaa

1901 aacaaattac aaaaattcaa aattttatcg attacgcgtc acgtgctagc tgcagtaacg ccattttgca aggcatggaa aaataccaaa ccaagaatag

2001 agaagttcag atcaagggcg ggtacatgaa aatagctaac gttgggccaa acaggatatc tgcggtgagc agtttcggcc ccggcccggg gccaagaaca

2101 gatggtcacc gcagtttcgg ccccggcccg aggccaagaa cagatggtcc ccagatatgg cccaaccctc agcagtttct taagacccat cagatgtttc

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7601 ccgagcgcag aagtggtcct gcaactttat ccgcctccat ccagtctatt aattgttgcc gggaagctag agtaagtagt tcgccagtta atagtttgcg

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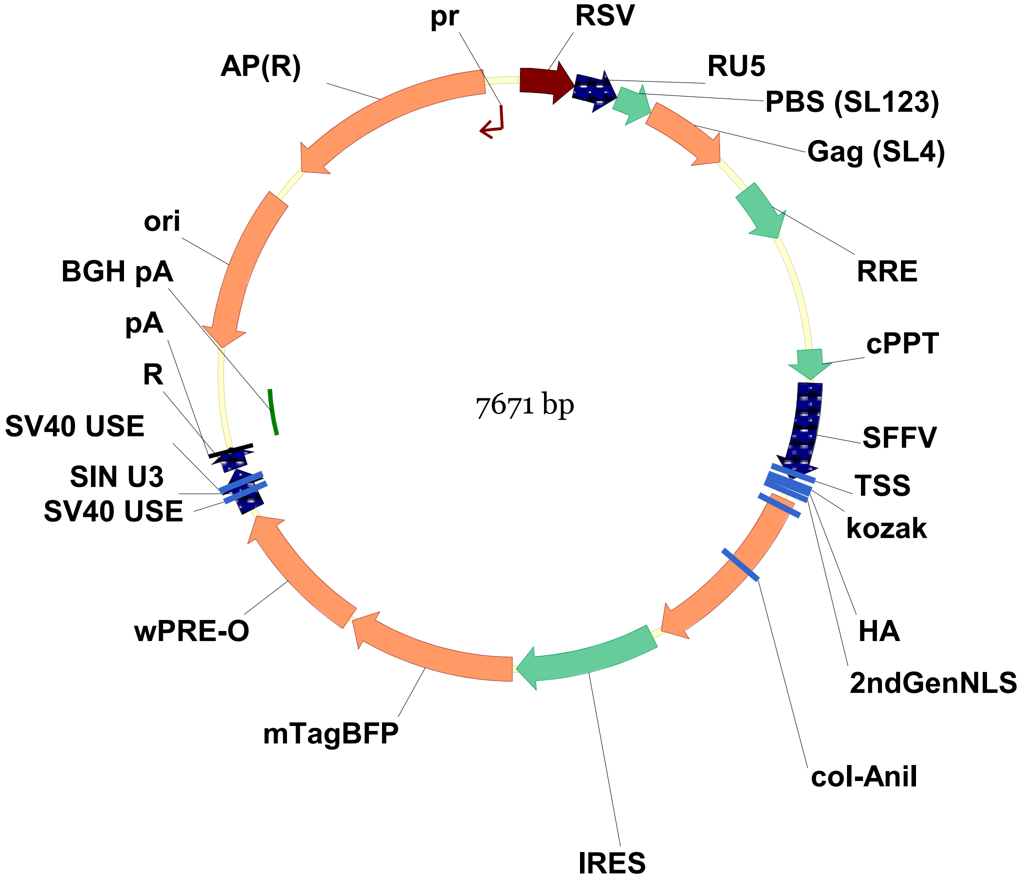

General Description

DNA Plasmid pCVL.SFFV.HA.NLS.I-Ani I.IRES.mTagBFP

Entire molecule length: 7671 bp

Feature Map

CDS (5 total)

Gag (SL4)

Start: 572   End: 924

Original Location Description:

572..924

coAnil

Start: 2438 End: 3199

Original Location Description:

2438..3199

mTagBFP

Start: 3844 End: 4545

Original Location Description:

3844..4545

wPRE-O

Start: 4560 End: 5140

Original Location Description:

4560..5140

AP(R)

Start: 6681 End: 7541 (Complementary)

bla gene- Ap(r) determinant

Original Location Description:

complement(6681..7541)

LTR (4 total)

RU5

Start: 236   End: 417

Original Location Description:

236..417

SFFV

Start: 1946 End: 2355

Original Location Description:

1946..2355

Qualifiers:

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/product="#AJ224005"

/SECDrawAs="Gene"

```
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    Start: 5172  End: 5353
    Original Location Description:
       5172..5353
  R
    Start: 5354  End: 5443
    Original Location Description:
       5354..5443
Misc. Binding Site (1 total)
  loxP
    Start: 5210  End: 5243
    Original Location Description:
       5210..5243
    Qualifiers:
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       /product="loxP"
Misc. Feature (10 total)
  PBS (SL123)
    Start: 418   End: 571
    Original Location Description:
       418..571
  RRE
    Start: 1081  End: 1322
    Original Location Description:
       1081..1322
  cPPT
    Start: 1806  End: 1929
    Original Location Description:
       1806..1929
  kozak
    Start: 2366  End: 2374
```

Original Location Description:

2366..2374

ATG

Start: 2375 End: 2377

Original Location Description:

2375..2377

HA

Start: 2381 End: 2407

Original Location Description:

2381..2407

2ndGenNLS

Start: 2408 End: 2431

Original Location Description:

2408..2431

Y13F

Y111S

IRES

Start: 3243 End: 3828

Original Location Description:

3243..3828

Misc. Marker (3 total)

junction

SV40 USE

Start: 5246 End: 5289

Original Location Description:

5246..5289

SV40 USE

Start: 5293 End: 5336

Original Location Description:

5293..5336

PolyA Signal (1 total)

BGH pA

Start: 5445 End: 5678

Original Location Description:

5445..5678

PolyA Site (1 total)

pA

Start: 5427 End: 5432

Original Location Description:

5427..5432

Primer (1 total)

CVL-34

Start: 1671 End: 1692

Original Location Description:

1671..1692

Promoter Prokaryotic (2 total)

RSV

Start: 7   End: 235

Original Location Description:

7..235

pr

Start: 7606 End: 7611 (Complementary)

Original Location Description:

complement(7606..7611)

Replication Origin (1 total)

ori

Start: 5860 End: 6533 (Complementary)

Original Location Description:

complement(5860..6533)

mRNA (1 total)

TSS

Start: 2330 End: 2355

Original Location Description:

2330..2355

Mutation (1 total)

A->G

Sequence

```
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 101 cgattggtgg aagtaaggtg gtacgatcgt gccttattag gaaggcaaca gacgggtctg acatggattg gacgaaccac tgaattgccg cattgcagag
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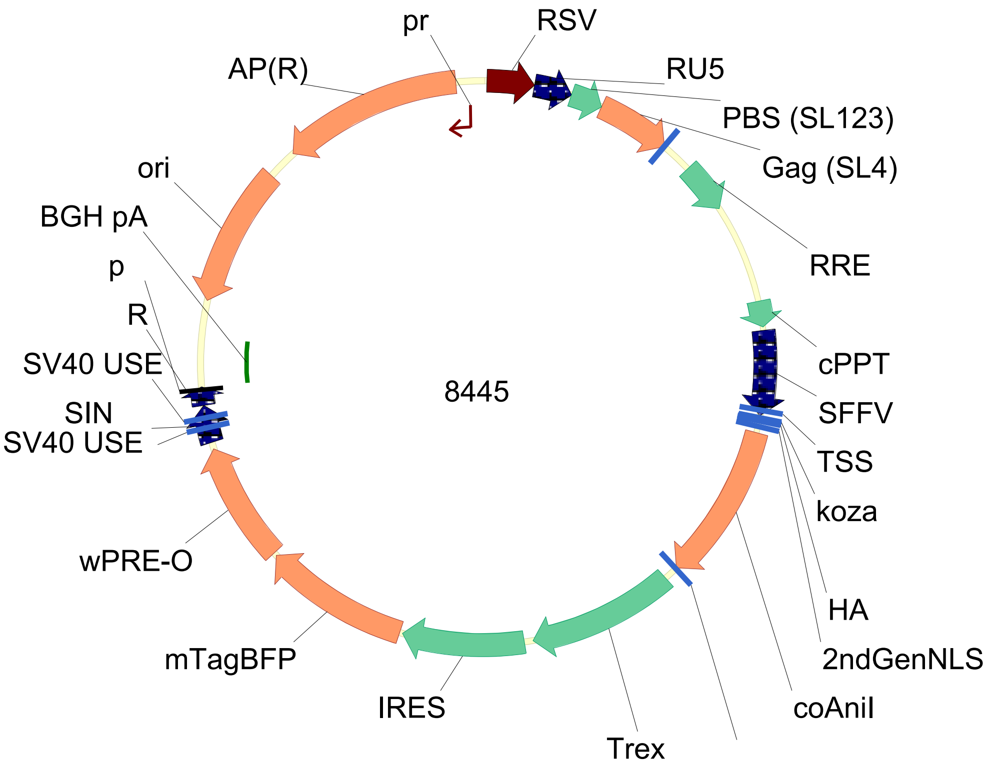

General Description

DNA Plasmid pCVL.SFFV.HA.NLS.IAni-I.T2A.Trex2.IRES.mTagBFP

Entire molecule length: 8445 bp

Feature Map

CDS (5 total)

Gag (SL4)

Start: 572   End: 924

Original Location Description:

572..924

coAniI

Start: 2438 End: 3199

Original Location Description:

2438..3199

mTagBFP

Start: 4618 End: 5319

Original Location Description:

4618..5319

wPRE-O

Start: 5334 End: 5914

Original Location Description:

5334..5914

AP(R)

Start: 7455 End: 8315 (Complementary)

bla gene- Ap(r) determinant

Original Location Description:

complement(7455..8315)

LTR (4 total)

RU5

Start: 236   End: 417

Original Location Description:

236..417

SFFV

Start: 1946 End: 2355

Original Location Description:

1946..2355

Qualifiers:

/gene="'MESV"

/product="#AJ224005"

/SECDrawAs="Gene"

SIN U3

Start: 5946 End: 6127

Original Location Description:

5946..6127

R

Start: 6128 End: 6217

Original Location Description:

6128..6217

Misc. Binding Site (1 total)

loxP

Start: 5984 End: 6017

Original Location Description:

5984..6017

Qualifiers:

/gene="(null)"

/product="loxP"

Misc. Feature (13 total)

PBS (SL123)

Start: 418   End: 571

Original Location Description:

418..571

RRE

Start: 1081 End: 1322

Original Location Description:

1081..1322

cPPT

Start: 1806 End: 1929

Original Location Description:

1806..1929

kozak

Start: 2366 End: 2374

Original Location Description:

2366..2374

ATG

Start: 2375 End: 2377

Original Location Description:

2375..2377

HA

Start: 2381 End: 2407

Original Location Description:

2381..2407

2ndGenNLS

Start: 2408 End: 2431

Original Location Description:

2408..2431

Y13F

Y111S

Trex2

Start: 3269 End: 3976

Original Location Description:

3269..3976

K77 (to A = dimeriziation defect (K59A in human))

D193 (to N = catalytically inactive dominate negative)

IRES

Start: 4017 End: 4602

Original Location Description:

4017..4602

Misc. Marker (3 total)

junction

Start: 927 End: 930

Original Location Description:

927..930

SV40 USE

Start: 6020 End: 6063

Original Location Description:

6020..6063

SV40 USE

Start: 6067 End: 6110

Original Location Description:

6067..6110

PolyA Signal (1 total)

BGH pA

Start: 6219 End: 6452

Original Location Description:

6219..6452

PolyA Site (1 total)

pA

Start: 6201 End: 6206

Original Location Description:

6201..6206

Primer (1 total)

CVL-34

Start: 1671 End: 1692

Original Location Description:

1671..1692

Promoter Prokaryotic (2 total)

RSV

Start: 7 End: 235

Original Location Description:

7..235

pr

Start: 8380 End: 8385 (Complementary)

Original Location Description:

complement(8380..8385)

Replication Origin (1 total)

ori

Start: 6634 End: 7307 (Complementary)

Original Location Description:

complement(6634..7307)

mRNA (1 total)

TSS

Start: 2330 End: 2355

Original Location Description:

2330..2355

Mutation (1 total)

Misc. Signal (1 total)

T2A

Start: 3203 End: 3262

Original Location Description:

3203..3262

Sequence

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101 cgattggtgg aagtaaggtg gtacgatcgt gccttattag gaaggcaaca gacgggtctg acatggattg gacgaaccac tgaattgccg cattgcagag

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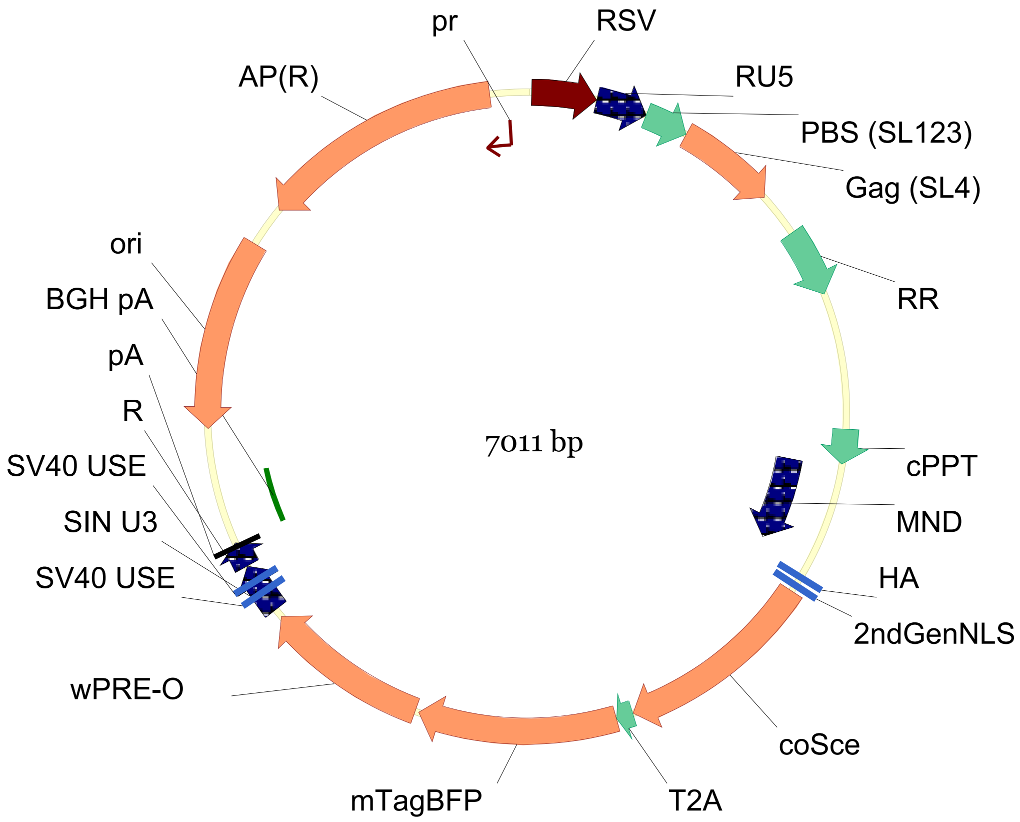

General Description

DNA Plasmid (#906) pCVL.MND.SceOPT.2A.TagBFP

Entire molecule length: 7011 bp

Feature Map

CDS (5 total)

Gag (SL4)

Start: 572 End: 924

Original Location Description:

572..924

coSce

Start: 2410 End: 3120

Original Location Description:

2410..3120

mTagBFP

Start: 3184 End: 3885

Original Location Description:

3184..3885

wPRE-O

Start: 3900 End: 4480

Original Location Description:

3900..4480

AP(R)

Start: 6021 End: 6881 (Complementary)

bla gene- Ap(r) determinant

Original Location Description:

complement(6021..6881)

LTR (3 total)

RU5

Start: 236   End: 417

Original Location Description:

236..417

SIN U3

Start: 4512 End: 4693

Original Location Description:

4512..4693

R

Start: 4694 End: 4783

Original Location Description:

4694..4783

Misc. Binding Site (1 total)

Misc. Feature (5 total)

PBS (SL123)

Start: 418   End: 571

Original Location Description:

418..571

RRE

Start: 1081 End: 1322

Original Location Description:

1081..1322

cPPT

Start: 1806 End: 1929

Original Location Description:

1806..1929

HA

Start: 2353 End: 2379

Original Location Description:

2353..2379

2ndGenNLS

Start: 2386 End: 2409

Original Location Description:

2386..2409

Misc. Marker (3 total)

junction

SV40 USE

Start: 4586 End: 4629

Original Location Description:

4586..4629

SV40 USE

Start: 4633 End: 4676

Original Location Description:

4633..4676

PolyA Signal (1 total)

BGH pA

Start: 4785 End: 5018

Original Location Description:

4785..5018

PolyA Site (1 total)

pA

Start: 4767 End: 4772

Original Location Description:

4767..4772

Promoter Prokaryotic (2 total)

RSV

Start: 7   End: 235

Original Location Description:

7..235

pr

Start: 6946 End: 6951 (Complementary)

Original Location Description:

complement(6946..6951)

Replication Origin (1 total)

ori

Start: 5200 End: 5873 (Complementary)

Original Location Description:

complement(5200..5873)

Repeat Region (1 total)

MND

Start: 1942 End: 2288

Original Location Description:

1942..2288

Mutation (2 total)

Misc. Signal (1 total)

T2A

Start: 3124 End: 3183

Original Location Description:

3124..3183

Sequence

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101 cgattggtgg aagtaaggtg gtacgatcgt gccttattag gaaggcaaca gacgggtctg acatggattg gacgaaccac tgaattgccg cattgcagag

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