TagRFP Simplicon™ Plasmid (E3L)

Plasmid DNA

Cat. # SCR726

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Pack size: 10 µg

Store at -20 ºC

Data Sheet

Background

Simplicon™ is a novel system to effect immediate high sustained protein expression of multiple genes into transfected cells without the risk of genome integration. The technology employs a single, synthetic, polycistronic, self-replicating RNA based on the Venezuelan equine encephalitis (VEE) genome[1,2,3,4]. The Simplicon™ RNA contains only genes encoding the VEE RNA replication machinery while the structural proteins that are required to make an infectious particle have been removed and replaced with the transgenes of interest. The Simplicon™ RNA is a synthetic RNA generated from the Simplicon™ Cloning Vector (E3L) plasmid.

Introduction and replication of the Simplicon™ RNA is expected to elicit a strong interferon response in transfected cells. To suppress the IFN responses, a Vaccinia virus protein[5], B18R, is used for the original Simplicon™ technology. Recently, we found that another Vaccinia virus protein[5], E3L, also suppresses the IFN responses in Simplicon™ RNA expression. B18R neutralizes type I interferons by direct binding, while E3L inhibits the cytoplasmic signaling pathways of IFN responses. Therefore, B18R and E3L are both employed in the Simplicon™ Expression System and work collaboratively to suppress IFN responses. As a result, there is increased cell viability during RNA transfection and increased expression of the transgenes. The Simplicon™ Expression System works in human cells and is not expected to work in mouse cells. This is because the B18R does not effectively neutralize mouse interferon (IFN)-β.

One day after transfection of the Simplicon™ RNA, a spike in the levels of transgenes can be observed. The expression levels are maintained by addition of B18R, E3L and the selective agent, puromycin throughout the duration of the experiment. Over time, expression levels are expected to diminish and stabilize to 1/5 – 1/10 the levels initially observed and may be close to physiological levels after one week. Expression levels and duration may change depending upon the cell types, transgenes and media conditions used. The Simplicon™ technology has been successfully utilized for efficient human iPSC generation through the sustained expression of critical reprogramming factors[3,4] and in the creation of cell lines that express and retain the metabolic activities of five cytochrome P450 enzymes[6].

In the Simplicon™ Expression System, B18R and E3L are provided as a B18R-E3L RNA (Cat. No. SCR722) for the suppression of IFN responses at RNA transfection. For sustained transgene expression, recombinant B18R protein (Cat. No. SCR156 and SCR197) or B18R conditioned medium (B18R-CM) can be used. E3L is continuously provided from the Simplicon™ RNA itself. B18R-CM can be produced from B18R-E3L RNA or B18R RNA synthesized using the B18R-E3L plasmid (Cat. No. SCR727) or B18R plasmid (Cat. No. SCR728), respectively.

The TagRFP Simplicon™ Plasmid (E3L) was developed for the synthesis of TagRFP Simplicon™ RNA (E3L). The Simplicon™ TagRFP RNA may be used to determine optimal transfection conditions to express the self-replicating RNA in hard-to transfect somatic or primary cells. Simplicon™ TagRFP expresses a monomeric red (orange) fluorescent protein generated from the RFP of sea anemone *Entacmaea quadricolor*. TagRFP exhibits fluorescence with excitation/emission maxima at 555/584 nm respectively, and brightness that is nearly three times higher than mCherry[7,8].

Plasmid Information

Plasmid map is indicated on the next page. Full DNA sequence data is available on our website (www.emdmillipore.com).

Transformation and Amplification of Plasmid

DH5α, DH10B or equivalent competent cells may be used for the transformation and amplification of the plasmids.

RNA Synthesis

The complete protocol for cloning and RNA synthesis is available in the User Guide for Simplicon™ System on our website (www.emdmillipore.com).

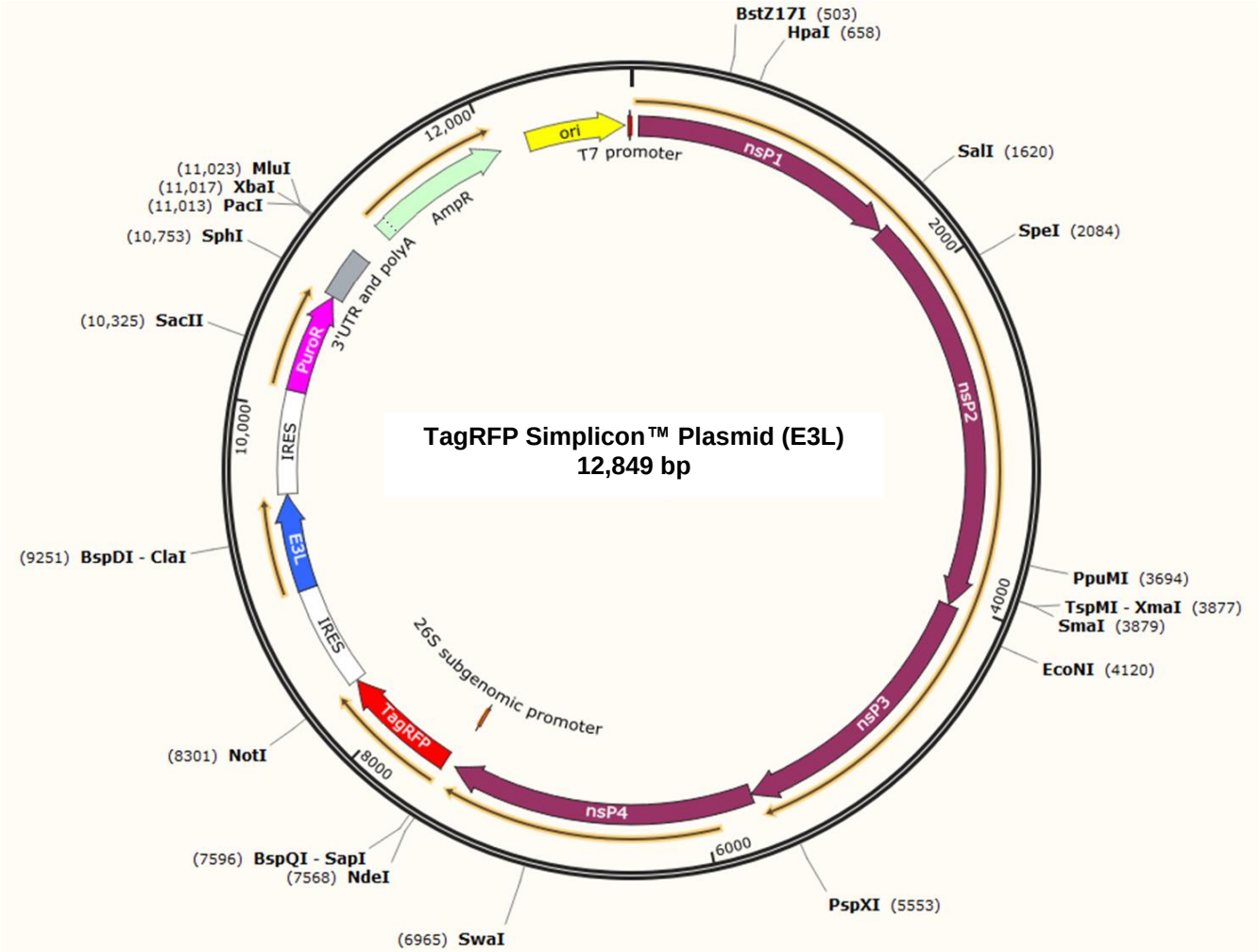
Storage & Stability:

TagRFP Simplicon™ Plasmid (E3L): (CS224505) One (1) vial containing 10 µL of DNA (1 µg/µL). Store at -20 °C.

References

1. Petrakova O, et al. 2005 *J Virol* **79**(12): 7597-7608.
2. Zimmer G 2010 *Viruses* **2**(2): 413-434.
3. Yoshioka N, et al. 2013 *Cell Stem Cell*. **13** (2): 246-254.
4. Yoshioka N, et al. 2017 *PLoS One*. **12**(7): e0182018.
5. Perdiguero B, et al. 2009 *J Interferon Cytokine Res*. **29**(9): 581-98.
6. Pegg G, et al. 2018 *Drug Metabolism and Pharmacokinetics* 33 (1): S33-S34.
7. Mertzlyak EM, et al. 2007 *Nat. Methods* 4: 555-557.
8. Subach OM, et al. 2008 *Chem. Biol.* 15: 1116-1124.

Rev.1.0/2018-04-20/SCR726/VC



T7 promoter: Partial promoter for bacteriophage T7 RNA polymerase. Allows *in vitro* transcription of the Simplicon™ RNA.

Non-structural genes (nsP1-4): Encodes four nonstructural proteins (nsP1-4) that are responsible for replication of Simplicon™ RNA (genomic RNA) and transcription of subgenomic RNA (your genes, E3L and puromycin).

26S Subgenomic Promoter: Promotes the transcription of subgenomic RNAs with nsP proteins.

TagRFP: Encodes TagRFP gene.

IRES: Internal Ribosome Entry Site. Allows for translation of E3L and Puromycin genes.

E3L: Encodes Vaccinia virus E3L gene. Human codon optimized.

PuroR: Encodes puromycin resistance gene. Confers resistance to puromycin.

3' UTR: Partial 3' UTR from VEE RNA replicon.

Poly (A): Long poly (A) tail (175 nucleotides) is incorporated into the vector and thus the poly (A) adenylation reaction is no longer required.

AmpR: Ampicillin resistance gene. Confers resistance to ampicillin in *E coli*.

Ori: high-copy-number ColE1/pMB1/pBR322/pUC origin of replication in *E. coli*.

Full DNA sequences are available from our website: www.emdmillipore.com

RELATED PRODUCTS

Cat #	Description
SCR720	TagGFP2 Simplicon™ RNA (E3L) Kit
SCR721	TagRFP Simplicon™ RNA (E3L) Kit
SCR722	B18R-E3L RNA (human codon optimized for B18R and E3L)
SCR723	B18R RNA (human codon optimized)
SCR724	Simplicon™ Cloning Vector (E3L)
SCR725	TagGFP2 Simplicon™ Plasmid (E3L)
SCR727	B18R-E3L Plasmid (human codon optimized fro B18R and E3L)
SCR728	B18R Plasmid (human codon optimized)
SCR729	Human OKSG-cMyc TagRFP Simplicon™ Plasmid
GF156	B18R protein (produced from insect)
GF197	B18R protein (priduced from HEK 293 cells)

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"Product" means TagRFP Simplicon™ Plasmid (E3L) (SCR726)

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