B18R-E3L Plasmid (human codon optimized for B18R and E3L)

Plasmid DNA

Cat. # SCR727

Pack size: 10 µg

Store at -20 ºC

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

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 B18R-E3L Plasmid (human codon optimized for B18R and E3L (Cat. No. SCR727) is used as a DNA template for the synthesis of B18R-E3L RNA (Cat. No. SCR722). The B18R-E3L RNA is a synthetic polycistronic mRNA and is used for co-transfected with the Simplicon™ RNA to suppress the IFN responses. B18R-E3L RNA can also be used to prepare B18R-CM by transfection into HFFs (Cat. No. SCC058). B18R-E3L RNA is also available for co-transfection of any kinds of mRNA to suppress the IFN responses. Please refer to the User Guide for Simplicon™ Expression System located on our website (www.emdmillipore.com) for detailed protocols.

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™ Expression System on our website (www.emdmillipore.com).

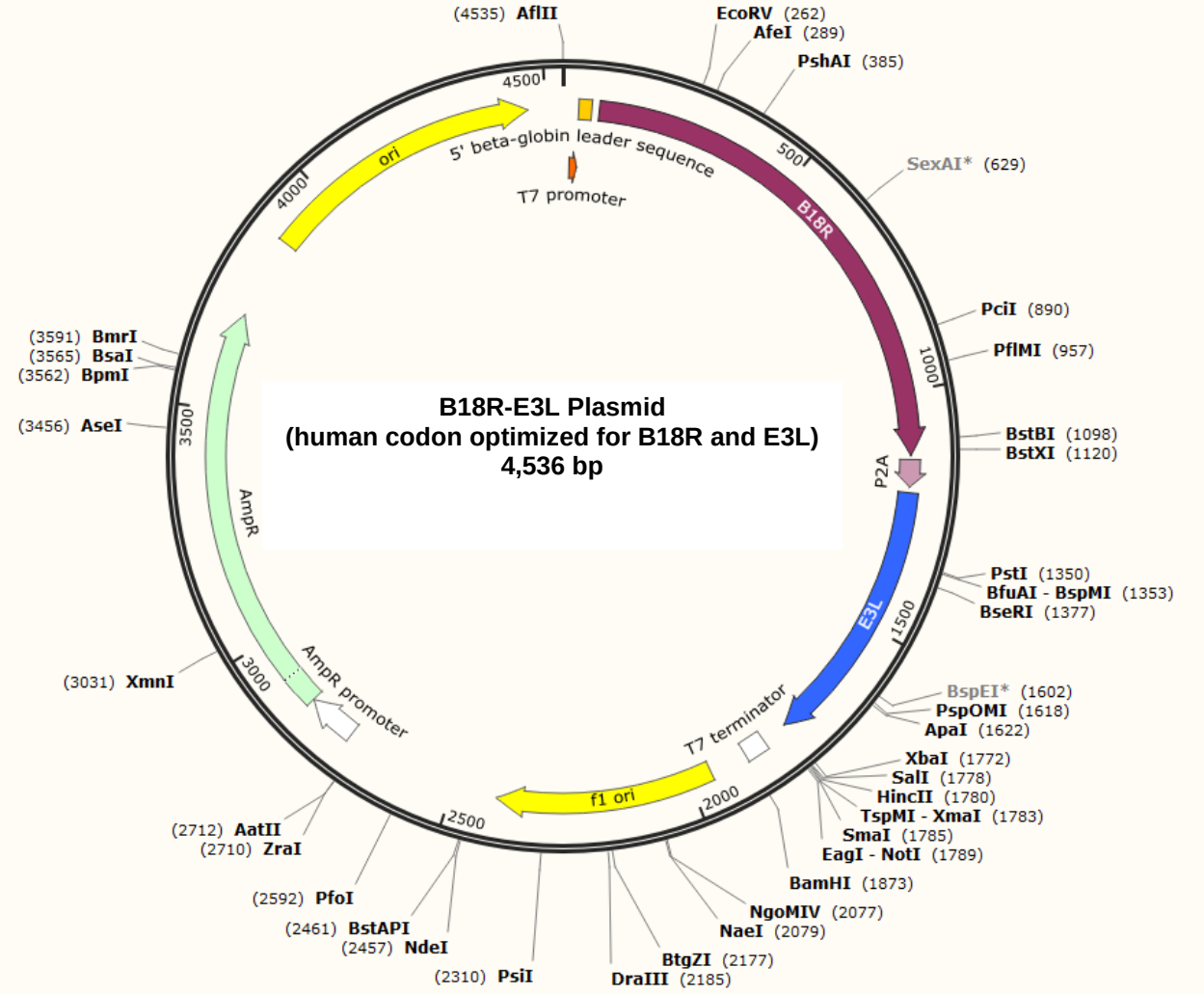
Storage & Stability of Component

B18R-E3L Plasmid (human codon optimized for B18R and E3L) (CS224506) 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.*, *Cell Stem Cell*. **13** (2): 246-254.
4. Yoshioka N, Dowdy SF (2017), *PLoS One*. **12**(7): e0182018.
5. Perdiguero B, Esteban M. (2009), *J Interferon Cytokine Res*. **29**(9): 581-98.
6. Pegg G., *et al*. (2018), Drug Metabolism and Pharmacokinetics 33 (1): S33-S34.

Rev.1.0/2018-04-20/SCR727/VC



T7 promoter: Minimum Promoter for bacteriophage T7 RNA polymerase. Allows *in vitro* transcription of the B18R-E3L-RNA.
5´ β-globin leader sequence: Increases the translation of several genes for more rapid initiation of translation.
B18R: Encodes Vaccinia virus B18R gene. Human codon optimized.
P2A: P2A peptide from porcine teschovirus-1 polyprotein. Allows the polycistronic expression of B18R and E3L proteins.
E3L: Encodes Vaccinia virus E3L gene. Human codon optimized.
T7 terminator: transcription terminator for bacteriophage T7 RNA polymerase
Poly (A) tail: 30 bases of poly A tail has been added in plasmid backbone. 30 bases of poly A is not enough length to stabilize the RNA. Poly (A) adenylation reaction is 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)
SCR726	TagRFP Simplicon™ Plasmid (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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(subject to local law)

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"Product" means B18R-E3L Plasmid (human codon optimized for B18R and E3L) (SCR727)

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