

B18R Plasmid (human codon optimized)

Plasmid DNA

Cat. # SCR728

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NOT FOR USE IN DIAGNOSTIC PROCEDURES.
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Pack size: 10 µg

Store at -20 °C



Data Sheet

page 1 of 2

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 protein⁵, B18R, is used for the original Simplicon™ technology.

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 enzymes⁶.

In the Simplicon™ Expression System, B18R is provided as a B18R 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. B18R-CM can be produced from B18R RNA synthesized using B18R plasmid (Cat. No. SCR728), respectively.

The B18R Plasmid (human codon optimized) (Cat. No. SCR728) is used as a DNA template for the synthesis of B18R RNA (Cat. No. SCR722). The B18R RNA is a synthetic mRNA and is used for co-transfected with the Simplicon™ RNA. B18R RNA can be used to prepare B18R-CM by transfection into HFFs (Cat. No. SCC058). B18R 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 Plasmid (human codon optimized) (CS224508) One (1) vial containing 10 µL of DNA (1 µg/µL). Store at -20 °C.

References

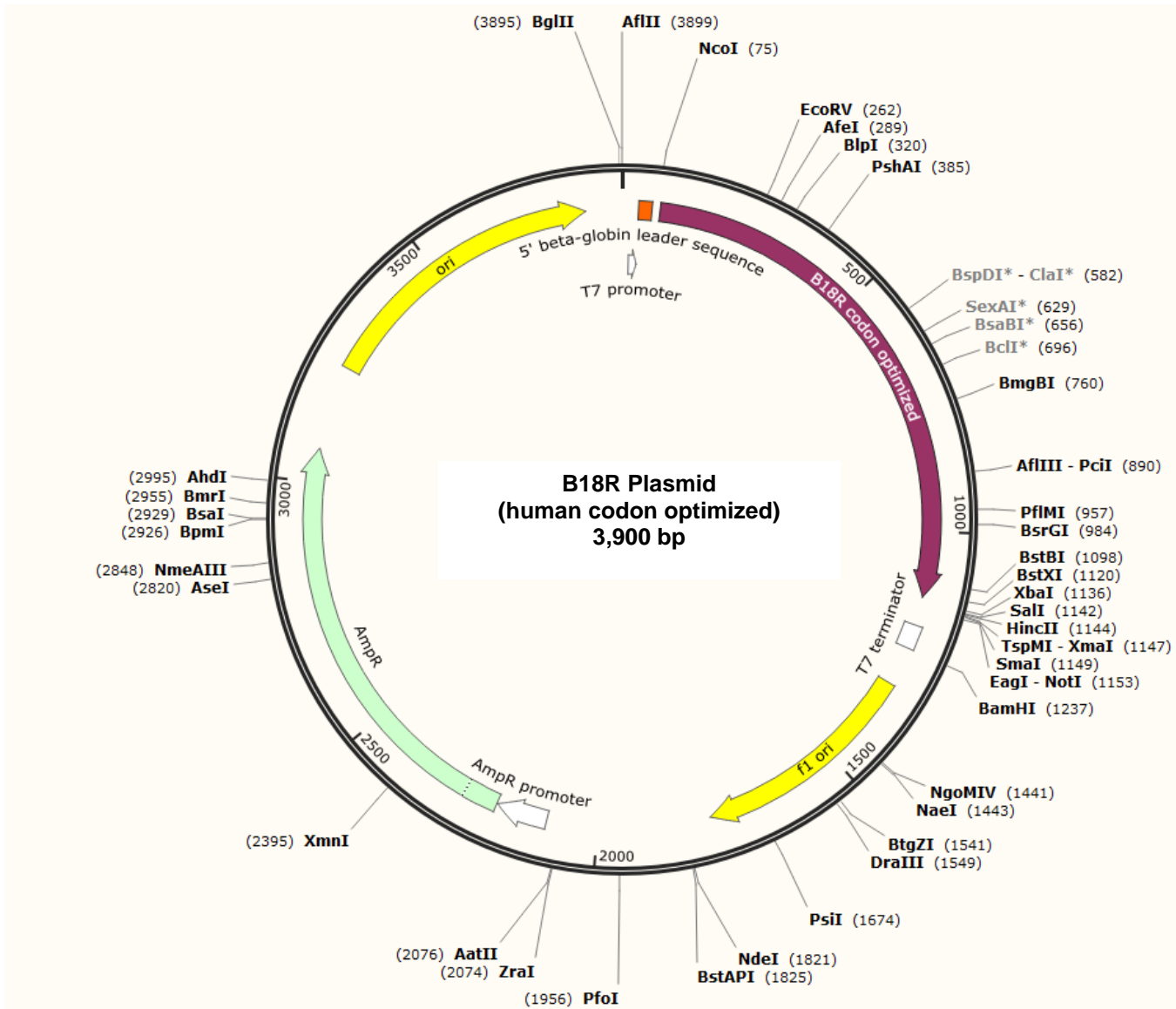
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3. Yoshioka N, et al., *Cell Stem Cell*. **13** (2): 246-254.
4. Yoshioka N, Dowdy SF (2017), *PLoS One*. **12**(7): e0182018.
5. Perdiguero B, Esteban M. (2009), *J Interferon Cytokine Res*. **29**(9): 581-98.
6. Pegg G., et al. (2018), *Drug Metabolism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

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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.

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 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

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RELATED PRODUCTS

Cat #	Description
SCR720	■ TagGFP2 Simplicon™ RNA (E3L) Kit
SCR721	■ TagRFP Simplicon™ RNA (E3L) Kit
SCR722	■ B18R-E3L RNA (human codon optimized fro B18R and E3L)
SCR723	■ B18R RNA (human codon optimized)
SCR724	■ Simplicon™ Cloning Vector (E3L)
SCR725	■ TagGFP2 Simplicon™ Plasmid (E3L)
SCR726	■ TagRFP Simplicon™ Plasmid (E3L)
SCR727	■ B18R-E3L Plasmid (human codon optimized for B18R and E3L)
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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