

Human OKSG-cMyc TagRFP Simplicon™ Plasmid

Plasmid DNA

Cat. # SCR729

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NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Pack size: 10 µg

Store at -20 °C



Data Sheet

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Background

Human OKSG-cMyc TagRFP Simplicon™ Plasmid is a DNA plasmid used to synthesize the self-replicating OKSG-cMyc TagRFP Simplicon™ RNA (Cat. No. SCR714) that is frequently used for reprogramming human somatic cells^{1,2}.

The Simplicon™ technology utilizes a single self-replicating Venezuelan equine encephalitis (VEE) RNA species that express the reprogramming factors (RF) ORFs (OKSG-cMyc; Oct4, Klf4, Sox2, Glis1 and cMyc)^{1,2} along with a red fluorescent protein (TagRFP)^{3,4}. The Simplicon™ RNA replicon is a synthetic polycistronic VEE RNA that is capable of self-replicating for a limited number of cell divisions. The OKSG-cMyc transgenes are especially useful for iPSCs generation from somatic cells that are more difficult to reprogram (i.e. slower proliferating cells or aged somatic cells)² while the TagRFP provides a rapid assessment of transfection efficiency. The presence of the TagRFP transgene also allows for optimization of the transfection conditions in hard to transfect somatic or primary cells. TagRFP is 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^{3,4}.

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. Recently, we found that another Vaccinia virus protein⁵, 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 OKSG-cMyc TagRFP Simplicon™ RNA, a spike in the level of TagRFP can be observed. The expression levels are maintained by addition of B18R and the selective agent, puromycin throughout the duration of the experiment. Expression levels and duration may change depending upon the cell types and media condition used.

Please note that the Human OKSG-cMyc TagRFP Simplicon™ Plasmid does not contain E3L, so E3L is not produced by OKSG-cMyc TagRFP Simplicon™ RNA. B18R-E3L RNA (Cat. No. SCR722) can be used to increase the viability and expression of the RFs by co-transfection with OKSG-cMyc TagRFP Simplicon™ RNA.

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 plasmid.

RNA Synthesis

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

iPSC Generation

Detailed protocols for iPSC generation is available in the user manual for the OKSG-cMyc TagRFP Simplicon™ RNA (Cat. No. SCR714).

Storage & Stability of Component

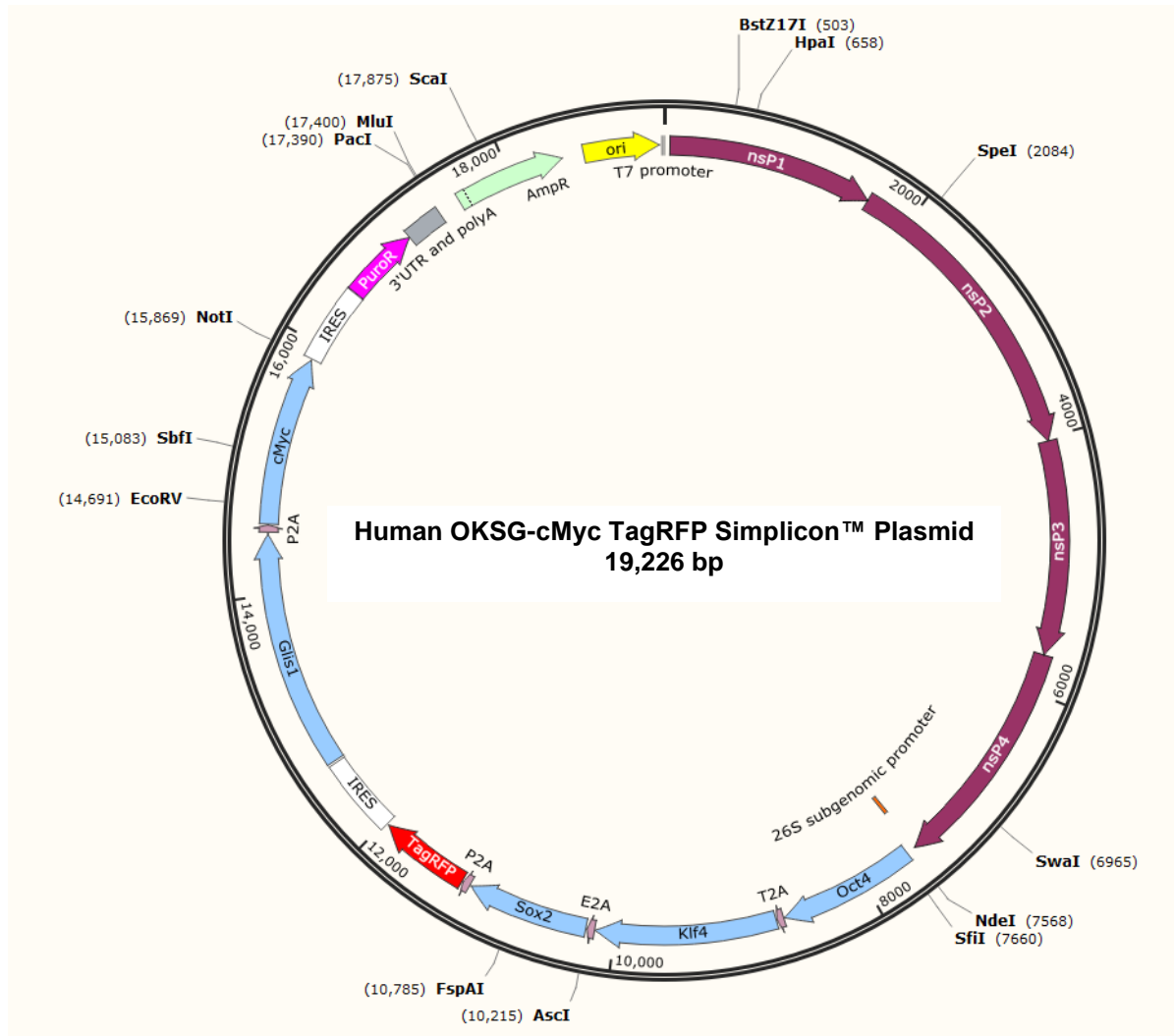
Human OKSG-cMyc TagRFP Simplicon™ Plasmid: (CS224507)
One (1) vial containing 10 µL of DNA (1 µg/µL). Store at -20 °C.

References

1. Yoshioka N, et al. 2013 *Cell Stem Cell* **13**(2): 246-254.
2. Yoshioka N and Dowdy SF. 2017 *PLoS One* **12**(7): e0182018
3. Mertzlyak EM, et al. 2007 *Nat. Methods* **4**: 555-557.
4. Subach OM, et al. 2008 *Chem. Biol.* **15**: 1116-1124.
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.

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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 (OKSG-TagRFP and puromycin).

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

Oct4, Klf4, Sox2, Glis1 and cMyc: Reprogramming factors for human iPSCs.

TagRFP: a monomeric red (orange) fluorescent protein generated from the RFP of sea anemone *Entacmaea quadricolor*.

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

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RELATED PRODUCTS

Cat #	Description
SCR549	■ Simplicon™ Reprogramming RNA (OKSG)
SCR703	Human OKSG-cMyc Simplicon™ RNA
SCR714	Human OKSG-cMyc TagRFP Simplicon™ RNA
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)
SCR727	B18R-E3L Plasmid (human codon optimized for B18R and E3L)
SCR728	B18R Plasmid (human codon optimized)
GF156	B18R protein (produced from insect)
GF197	B18R protein (produced from HEK 293 cells)

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