CELLECTA

CRISPR sgRNA Cloning and Expression Vector pRSG21-U6-sg-CMV-TagGFP2-2A-Puro (linearized)

Product: pRSG21-U6-sg-CMV-TagGFP2-2A-Puro (linearized)

Catalog #: SVCRU621-L

Lot #: 180131008

Description:

The pRSG21-U6-sg-CMV-TagGFP2-2A-Puro sgRNA Cloning and Expression Vector is a human immunodeficiency virus (HIV) lentiviral vector with a constitutive U6 promoter to express sgRNA constructs and CMV promoter to express both TagGFP2 and puromycin-resistance gene. The vector was linearized by restriction digestion using BpiI (BbsI), agarose gel purified, and is ready for cloning sgRNA template oligos (guide + tracrRNA scaffold).

The pRSG21 Vector has the functional elements necessary for packaging into viral particles (when used with Cellecta's Ready-to-Use Packaging Mix, Cat.# CPCP-K2A, or most other second or third generation lentiviral packaging mixes), transduction, stable integration into genomic DNA, and expression of sgRNA constructs in target cells. 500 ng of BpiI (BbsI) linearized vector is provided, sufficient for 50 ligation reactions.

Fluorescence Marker: TagGFP2 (ex/em: 483/506nm)

Biosafety Level: BSL-2

Storage: -20°C

Shelf Life: 2 years from date of receipt with proper storage

Shipping Conditions: Blue Ice or Dry Ice

Contents:

#	Catalog #	Description
1	SVCRU621-L	**CRISPR sgRNA Cloning and Expression Vector** pRSG21-U6-sg-CMV-TagGFP2-2A-Puro (linearized) 500 ng; 10 ng/µl, 50 µl (50 reactions)

Quality Control:

1 µl of a control sgRNA template (20 µM each strand) was phosphorylated and annealed as described in the manual. 0.5 µl of phosphorylated, annealed control sgRNA template (0.2 µM), consisting of guide + tracrRNA scaffold, was ligated into 10 ng of pRSG21 vector at 16°C for 1 hour. After transformation, 90% of the clones contain control sgRNA insert based on the result of insert amplification with forward and reverse PCR primers.

PROTOCOLS

Please visit Cellecta's website for the latest protocols:
https://www.cellecta.com/resources/product-manuals-and-certificates/

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Safety Guidelines

The HIV-based lentivector system is designed to maximize its biosafety features, which include:

- A deletion in the enhancer of the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
- The RSV promoter upstream of 5'LTR in the lentivector allows efficient Tat-independent production of viral RNA, reducing the number of genes from HIV-1 that are used in this system.
- Number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev). The corresponding proteins are expressed from different plasmids lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector to prevent generation of recombinant replication-competent virus.
- None of the HIV-1 genes (gag, pol, rev) will be present in the packaged pseudoviral genome, as they are expressed from packaging plasmids lacking packaging signal—therefore, the lentiviral particles generated are replication-incompetent.
- Pseudoviral particles will carry only a copy of your expression construct.

Despite the above safety features, use of HIV-based vectors falls within NIH Biosafety Level 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus or the possibility of insertional mutagenesis. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at:

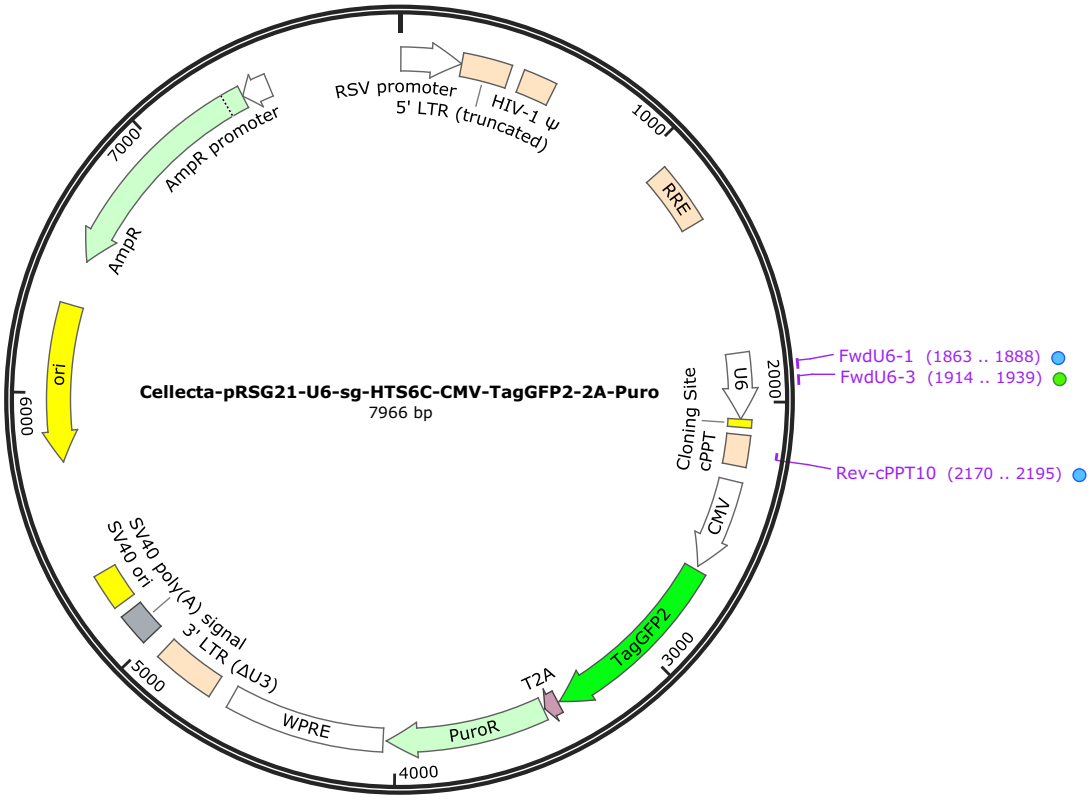
http://www.cdc.gov/biosafety/

It is also important to check with the health and safety guidelines at your institution regarding the use of lentiviruses and follow standard microbiological practices, which include:

- Wear gloves and lab coat at all times when conducting the procedure.
- Always work with pseudoviral particles in a Class II laminar flow hood.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (biohazard, infectious waste) container and sealed for transportation from the laboratory.

Appendix

1. Vector Map



2. Primers for Insert Screening

FwdU6-1	CAAGGCTGTTAGAGAGATAATTGGAA
Rev-cPPT10	TGTATGTCTGTTGCTATTATGTCTAC

3. Sanger Sequencing Primer

FwdU6-3	ATTAGTACAAAATACGTGACGTAGAA

For detailed vector maps, sequences, GenBank files, and CRISPR cassette designs, please visit https://www.cellecta.com/resources/vector-information/

For all other vectors, please contact Cellecta at tech@cellecta.com.

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