

Product Analysis Certificate

CRISPR sgRNA Cloning and Expression Vector
pRSGT17-U6Tet-sg-CMV-TetRep-2A-TagGFP2-2A-Puro (linearized)
Cat.# SVCRU6T17-L



CRISPR sgRNA Cloning and Expression Vector pRSGT17-U6Tet-sg-CMV-TetRep-2A-TagGFP2-2A-Puro (linearized)

Product: pRSGT17-U6Tet-sg-CMV-TetRep-2A-TagGFP2-2A-Puro (linearized)

Catalog #: SVCRU6T17-L

Lot #: 17040701

Description:

The pRSGT17-U6Tet-sg-CMV-TetRep-2A-TagGFP2-2A-Puro sgRNA Cloning and Expression Vector is a human immunodeficiency virus (HIV) lentiviral vector with an inducible U6-Tet promoter to express sgRNA constructs. The vector was linearized by restriction digestion using Bpil (BbsI), agarose gel purified, and is ready for cloning sgRNA template oligos (guide + tracrRNA scaffold).

The pRSGT17 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. This kit contains 500 ng of Bpil (BbsI) linearized vector that will be 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	SVCRU6T17-L	CRISPR sgRNA Cloning and Expression Vector pRSGT17-U6Tet-sg-CMV-TetRep-2A-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 pRSGT17 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.

Product Analysis Certificate

CRISPR sgRNA Cloning and Expression Vector
pRSGT17-U6Tet-sg-CMV-TetRep-2A-TagGFP2-2A-Puro (linearized)
Cat.# SVCRU6T17-L



PROTOCOLS

Please visit Celecta's website for the latest protocols:
<https://www.celecta.com/resources/product-manuals-and-certificates/>

Technical Support

Phone: +1 (650) 938-3910
Toll-Free: +1 (877) 938-3910
Fax: +1 (650) 938-3911

E-mail:
Technical Support: tech@celecta.com
General Information: info@celecta.com
Sales: sales@celecta.com
Orders: orders@celecta.com

Blog: <https://www.celecta.com/company/blog-news/>

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.

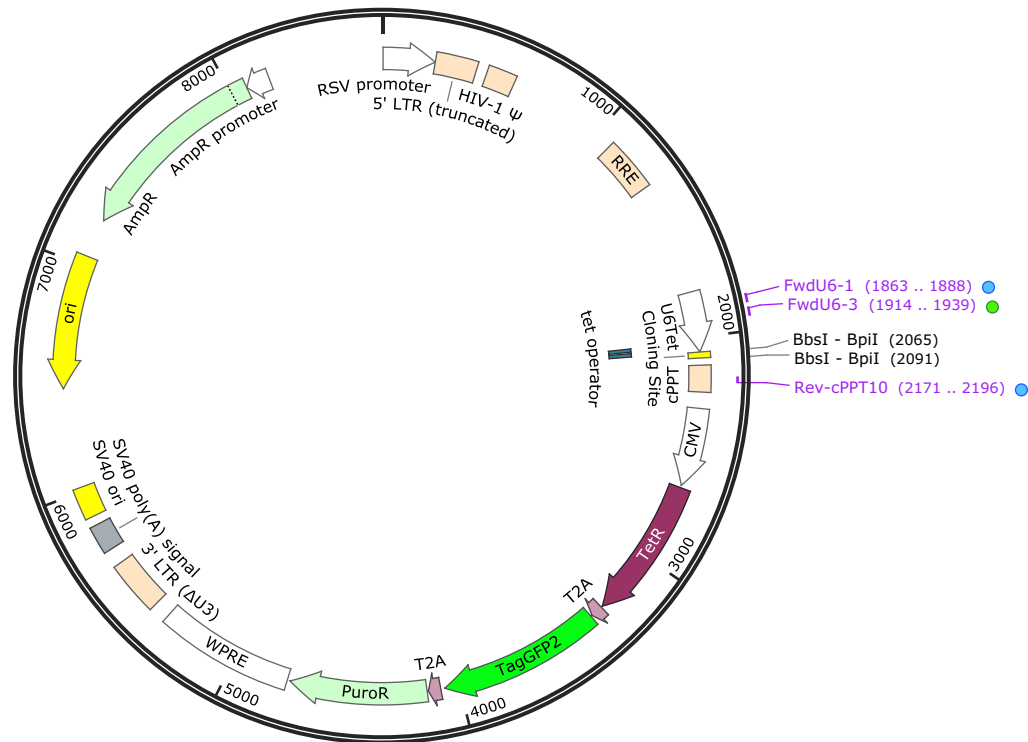
Product Analysis Certificate

CRISPR sgRNA Cloning and Expression Vector
pRSGT17-U6Tet-sg-CMV-TetRep-2A-TagGFP2-2A-Puro (linearized)
Cat.# SVCRU6T17-L



Appendix

1. Vector Map



Collecta-pRSGT17-U6Tet-sg-HTS6CTet-CMV-TetRep-2A-TagGFP2-2A-Puro
8676 bp

2. Primers for Insert Screening

FwdU6-1	CAAGGCTGTTAGAGAGATAATTGGAA
Rev-cPPT10	TGTATGTCTGTGCTATTATGTCTAC

3. Sanger Sequencing Primer

FwdU6-3	ATTAGTACAAAATACGTGACGTAGAA
---------	----------------------------

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

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

Product Analysis Certificate

CRISPR sgRNA Cloning and Expression Vector
pRSGT17-U6Tet-sg-CMV-TetRep-2A-TagGFP2-2A-Puro (linearized)
Cat.# SVCRU6T17-L



Terms and Conditions

Cellecta, Inc. Limited License

Cellecta grants the end user (the "Recipient") of the sgRNA Cloning and Expression Vector (the "Product") a non-transferable, non-exclusive license to use the reagents for internal research use only as described in the enclosed protocols; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Cellecta, Inc. -- separate licenses are available for non-research use or applications. The Product is not to be used for human diagnostics or included/used in any drug intended for human use. Care and attention should be exercised in handling the Product by following appropriate research laboratory practices.

Cellecta's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. Cellecta's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. Cellecta does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. Cellecta disclaims any and all responsibility for injury or damage that may be caused by the failure of the Recipient or any other person to use the Product in accordance with the terms and conditions outlined herein.

The Recipient may refuse these licenses by returning the enclosed Product unused. By keeping or using the enclosed Product, you agree to be bound by the terms of these licenses. The laws of the State of California shall govern the interpretation and enforcement of the terms of these Licenses.

Limited Use Licenses

The Recipient acknowledges that Product has been developed by Cellecta based on licenses from Third Parties and agrees with the Terms of Limited Use for the Recipient provided by the Third Parties:

Life Technologies Corporation End-User Label License for the use of Lentiviral Expression System:

"This product or service (based upon the Lentiviral Expression System) is sublicensed from Life Technologies Corporation under U.S. Patent Nos. 5,686,279; 5,834,256; 5,858,740; 5,994,136; 6,013,516; 6,051,427; 6,165,782; 6,218,187; 6,428,953; 6,924,144; 7,083,981 and 7,250,299 and corresponding patents and applications in other countries for internal research purposes only. Use of this technology for gene therapy applications or bioprocessing other than for nonhuman research use requires a license from GBP IP, LLC. Please contact GBP IP, LLC 537 Steamboat Road, Suite 200, Greenwich, CT 06830. Use of this technology to make or sell products or offer services for consideration in the research market requires a license from Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008."

Evrogen IP JSC End-User Label License for the use of lentiviral shRNA constructs comprising TagRFP-encoded gene:

"This product is for internal non-commercial research use only. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product. The right to use this product specifically excludes the right to validate or screen compounds. For information on commercial licensing, contact Evrogen Licensing Department, email: license@evrogen.com".

Terms and Conditions are also available online at <https://www.cellecta.com/company/legal-information/terms-and-conditions/>.

© 2017 Cellecta, Inc. All Rights Reserved.

Trademarks

CELLECTA is a registered trademark of Cellecta, Inc.