A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the License and Warranty Statement contained in this user manual.
## Contents

Product Description .............................................................................................................................................. 2
List of Components (Full Kit) .......................................................................................................................... 2
List of Components (Inducer Agent only) ......................................................................................................... 2
Storage ............................................................................................................................................................. 3
General Information .......................................................................................................................................... 3
Plasmid Vector Map ......................................................................................................................................... 3
Protocol for CLOuD9 Gene Expression Regulation Kit .................................................................................. 3
Example Data and Applications ...................................................................................................................... 5
References ......................................................................................................................................................... 6
Technical Support ............................................................................................................................................. 7
Licensing and Warranty Statement .................................................................................................................. 7
**Product Description**

SBI’s Chromatin Loop Reorganization using CRISPR-dCas9 (CLOuD9) kit represents an innovative approach to study the dynamic effects of chromatin restructuring on regulating gene expression in mammalian cells based on the paper “Manipulation of nuclear architecture through CRISPR-mediated chromosomal looping” (Morgan et al. 2017)¹.

Utilizing a novel application of the null nuclease (dCas9) and a modified plant-based chemical proximity sensor system (ABA pathway), the kit allows researchers to study the dynamic interplay between gene promoters and their control elements (e.g. enhancers and repressors) by promoting chromatin looping and juxtaposition of these regions to regulate gene expression. For the first time, researchers are able to study the effects of chromatin looping and its effects on gene expression in a convenient, plasmid-based system that is fully reversible, enabling increased understanding of chromatin biology in different cellular contexts.

The CLOuD9 kit is suitable for researchers who are interested in the role of chromatin in controlling gene expression in areas such as the following:

- Developmental biology
- Cancer biology
- Chromatin structural biology
- Chromatin-based therapeutic strategies

The CLOuD9 Gene Expression Regulation Kit (Cat #CASCL9-100A-KIT) comes with two separate (one spCas9-based and the other saCas9-based) dCas9-sensor fusion lentivector plasmids for cloning of specific gRNA sequences that allow targeting of the dCas9 to the genomic loci of interest. In addition, the 0.5M Inducer Agent (also sold separately, Cat #CASCL9-200A-1) will be provided in the kit to facilitate dimerization and chromatin looping.

**List of Components (Full Kit)**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>U6-gRNA scaffold-EF1a-dCas9-PYL1-P2A-Hygro lentiviral cloning vector</td>
<td>10 µg</td>
<td>-20°C</td>
</tr>
<tr>
<td>U6-gRNA scaffold-EF1a-dCas9-ABI-P2A-Puro lentiviral cloning vector</td>
<td>10 µg</td>
<td>-20°C</td>
</tr>
<tr>
<td>0.5M Inducer Agent</td>
<td>100 µl</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

**List of Components (Inducer Agent only)**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5M Inducer Agent</td>
<td>400 µl</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
Storage
The full kit and 0.5M Inducer Agent are shipped in blue ice and should be stored at -20°C. Properly stored kits are stable for 1 year from the date received.

General Information
The lentiviral plasmid DNA cloning vectors provided in the kit are 3rd-generation lentiviral vectors with self-inactivating (SIN) 3' LTR and hybrid LTR (CMV-5'LTR) features. We recommend propagating the plasmids using Stbl3 competent cells (Thermo Fisher Scientific Cat #C737303) according to the protocol provided by the manufacturer to reduce recombination of the plasmid and potential loss of sequences.

The plasmids are provided with a gRNA cloning site that allows for cloning of a single gRNA sequence compatible with spCas9 (dCas9-PYL1 plasmid) or saCas9 (dCas9-ABI plasmid). While the efficiency of spCas9 and saCas9 are similar, there are some key differences to consider in the design of the gRNAs.

1. Different PAM sequences:
   - spCas9 PAM: NGG
   - saCas9 PAMs: NNGGGT, NNGAAT, and NNGAGT (where “N” = any nucleotide)

2. saCas9 works most efficiently with gRNAs of 21 nt – 23 nt, spCas9 works most efficiently with gRNA of 20 nt

It may be useful to design gRNAs for the CLOuD9 system using several online gRNA design software available for both spCas9 and saCas9 (e.g. Broad’s sgRNA Designer for CRISPRko, amongst others) or commercially available software (e.g. Benchling’s cripsr tool, free for academic/non-profit)

Plasmid Vector Map

Protocol for CLOuD9 Gene Expression Regulation Kit

A. Cloning of the lentiviral vectors:
The full protocol for cloning into the lentiviral vectors can be found here:
https://media.addgene.org/data/plasmids/52/52963/52963-attachment_IPB7ZL_hjcbm.pdf

Note 1: You will need to order two sets (each set containing top and bottom oligos) for cloning into the two dCas9 lentiviral vectors in the CLOuD9 kit. Each set of oligos must be compatible with the PAM sequences of spCas9 or saCas9.

Note 2: There are two BsmBI sites in each plasmid which need to be removed for cloning. After removal by restriction digestion, you will generate a ~1.88kb, 14kb (spdCas9-PYL1) or 13kb (sadCas9-ABI) bands. You will need to gel purify the 13kb and 14kb bands for use as input for the gRNA cloning reaction.

B. Packaging of the lentiviral plasmids into lentivirus particles

A general protocol for virus packaging of the lentiviral vectors can be found in the Methods section (pg.8) of Morgan, SL, et al. Manipulation of nuclear architecture through CRISPR-mediated chromosomal looping. Nat Commun. 2017. Jul 13; 8:15993. Alternatively, SBI offers a full suite of virus packaging, concentrating, transduction, and titering reagents that are fully compatible with the plasmids in the kit.

Virus packaging plasmids:

pPACKH1 Lentiviral Packaging Mix (Cat #LV500-1): Contains GAG/POL, REV, and VSV-G plasmids in an optimized ratio for generating high-titer lentivirus particles.

Virus concentration reagents:

PEG-It Virus Concentration Reagent (Cat #LV810-1): 5X solution to effectively concentrate your virus particles in media to avoid large volume infection of target cells

Virus transduction reagents:

TransDux MAX Virus Transduction Reagent (Cat #LV860-1): Based on a proprietary formulation that is non-toxic and enables greater virus entry into target cells than standard Polybrene-based reagents.

Virus titering reagents:

Global UltraRapid Titering Kit (Cat #LV961-1): Get accurate functional virus titers using a qPCR-based system to measure pseudoviral particles that have integrated into target cells.

In general, we would recommend generating virus from 3-4 150mm plates (or equivalent surface area for flasks) for each plasmid construct to have virus of sufficient titer and volume for downstream infections of target cells.

C. Lentivirus Transduction Protocol

**Note 1:** The optimal concentration of Puro and Hygromycin will need to be determined for each cell line being tested. We recommend performing a kill curve for each non-infected cell line with different starting dosages of the antibiotics (e.g. 0.1 to 5ug/ml of Puro, 10 to 200ug/ml of Hygro) and determine the concentration that kills >90% of cells within 72hrs of drug administration. It is imperative to keep the cells in the selection media prior to performing the induction experiment.

**Note 2:** For non-toxic, efficient transduction of target cells, we would suggest the use of SBI’s TransDux MAX reagent as an alternative to Polybrene.

**Note 3:** The optimal MOI for infection of target cells will be determined by the cell’s ability to be successfully transduced. It may be useful to test target cells with a positive control GFP virus (SBI Cat #LV601B-1) to determine the approximate % of transduced cells by GFP expression, and using an MOI that shows ~40-50% infection of target cells.

**D. Induction Protocol**


**Note 1:** 0.5M Inducer Agent should be stored away from light, preferably at -20C. For short-term use, it can be stored at 4C away from light for one week.

**Note 2:** The amount of the inducing reagent will need to be tuned for the level of gene expression desired. The final concentration of the inducing reagent in the cells is 1 µM, however, higher amounts can be used for increased level of induction. DMSO can be used as the vehicle control.

**Note 3:** Other downstream applications such as ChIP-qPCR, ChIP-Seq, and ChIP-MS (mass spectrometry) may be performed to identify potential proteins involved in the chromatin looping process as well as confirming expression of the target gene being studies. For more information on these assays, please refer to the referenced paper.

**Example Data and Applications**
Figure 1. Schematic of the CLOuD9 system demonstrating chromatin looping upon addition of inducing reagent (ABA)

A. β-globin Expression

![β-globin Expression Graph](image)

B. Oct4 Expression (HEK293T Cells)

![Oct4 Expression Graph](image)

Figure 2: Representative induction data for CLOuD9 system at two different genetic loci

A. A CLOuD9-induced chromatin loop at the β-globin locus leads to activation of gene expression in K562 cells but not in HEK293T cells, highlighting the importance of cellular context and the need for additional factors in gene expression. Inducer reagent (in DMSO) was added for either 24-hours or 72-hours. At the indicated time, cells in the “inducer only” treatment group were harvested and analyzed for gene expression, whereas cells in the “inducer + washout” treatment group were washed and then allowed to grow another 24- or 72-hours as indicated in the absence of inducer. Significance assessed relative to DMSO-treated control cells which were not transduced with the CLOuD9 System. * indicates \( P < 0.05 \), *** indicates \( P < 0.0001 \).

B. Unlike the β-globin locus, a CLOuD9-induced chromatin loop (treated with Inducer Reagent in DMSO) results in robust expression of the Oct4 gene in CLOuD9/HEK293T cells compared to CLOuD9/HEK293T cells treated with DMSO only. The CLOuD9 System fusion proteins were targeted to the Oct4 promoter and the 5’ distal enhancer region upstream of the Oct4 promoter. * indicates \( P < 0.05 \).

References

Technical Support
For more information about SBI products and to download manuals in PDF format, please visit our web site: http://www.systembio.com

For additional information or technical assistance, please call or email us at:

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax: (650) 968-2277

E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

Licensing and Warranty Statement

Limited Use License

Use of the CLOuD9 Gene Expression Regulation Kit (i.e., the “Product”) is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

- The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.
- The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.
- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. 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. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI’s liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI’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. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose. SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.
This page intentionally left blank.
System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax: (650) 968-2277

E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com