CLOuD9™ Gene Expression Regulation Kit

Cat# CASCL9-100A-KIT

Cat# CASCL9-200A-1

User Manual

Store at -20^{0}C

Version 3
8/4/2017

Contents

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)

Item	Amount	Storage Temperature
U6-gRNA scaffold-EF1a-dCas9-PYL1-P2A-Hygro lentiviral cloning vector	10 µg	-20^{0}C
U6-gRNA scaffold-EF1a-dCas9-ABI-P2A-Puro lentiviral cloning vector	10 µg	-20^{0}C
0.5M Inducer Agent	100 µl	-20^{0}C

List of Components (Inducer Agent only)

Item	Amount	Storage Temperature
0.5M Inducer Agent	400 µl	-20^{0}C

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.

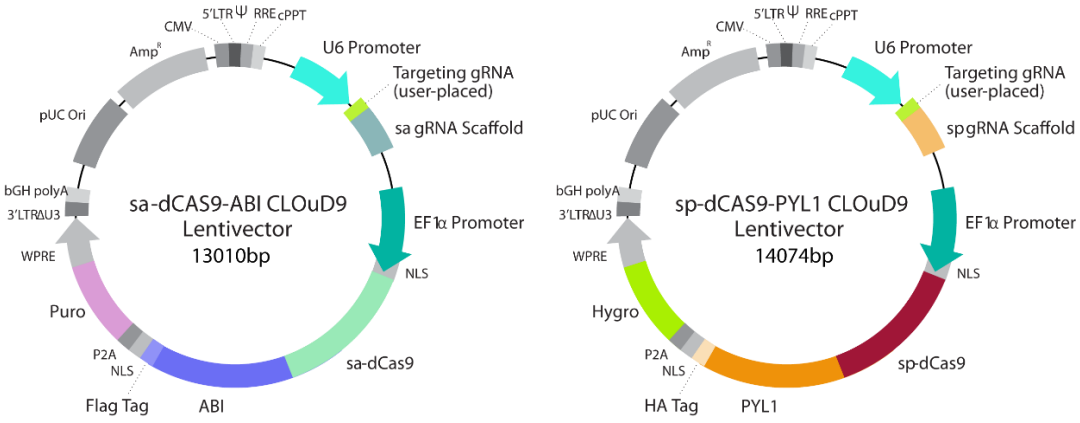
1. Different PAM sequences:

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 20nt

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 #LV500A-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 #LV810A-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 #LV860A-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 #LV961A-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

A general protocol for lentivirus transduction 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.

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

A general protocol for induction and washout of the inducing reagent 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.

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

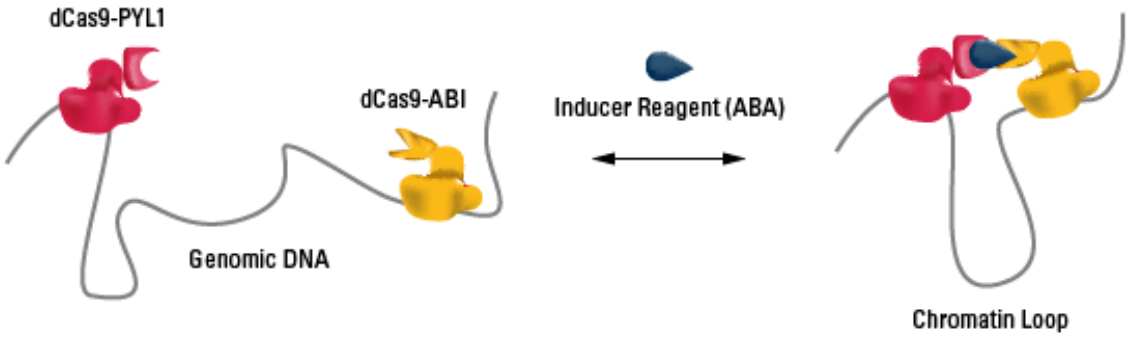


Figure 1. Schematic of the CLOuD9 system demonstrating chromatin looping upon addition of inducing reagent (ABA)

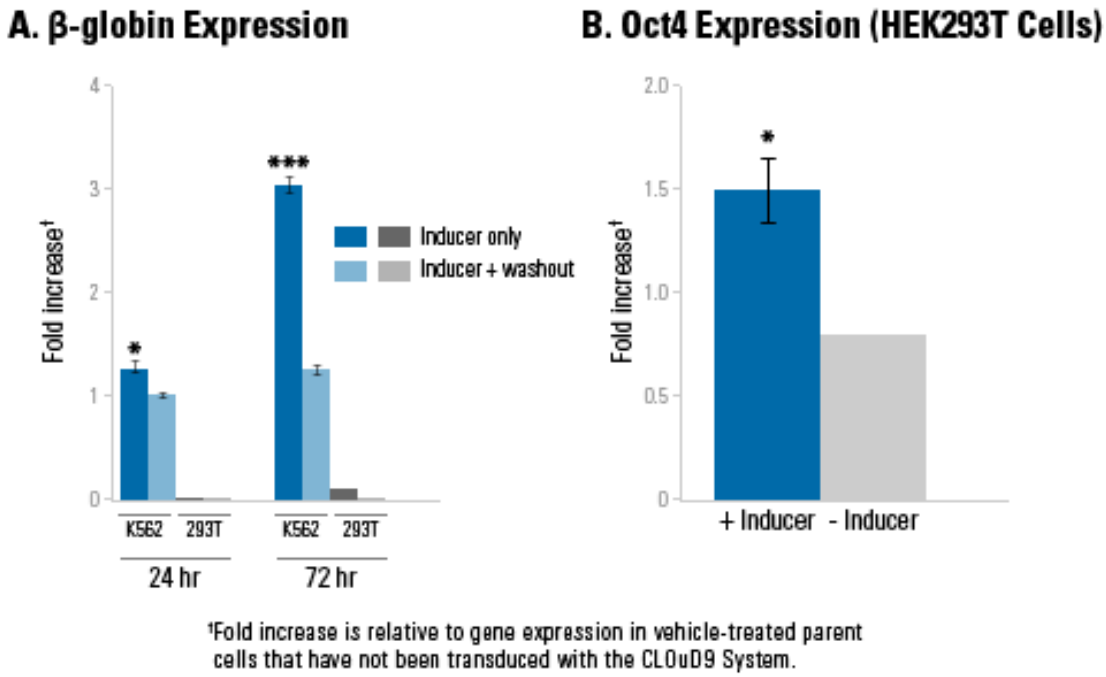


Figure 2: Representative induction data for CLOuD9 system at two different genetic loci

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

Morgan SL, *et al*. Manipulation of nuclear architecture through CRISPR-mediated chromosomal looping. *Nat Commun*. 2017. Jul 13; **8**:15993.

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:

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Licensing and Warranty Statement

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