SparQ™2 Cumate Switch System

Cat # QM82XB-1

User Manual

Please see individual components for storage conditions

Version 1
4/14/2023

Contents

Product Description

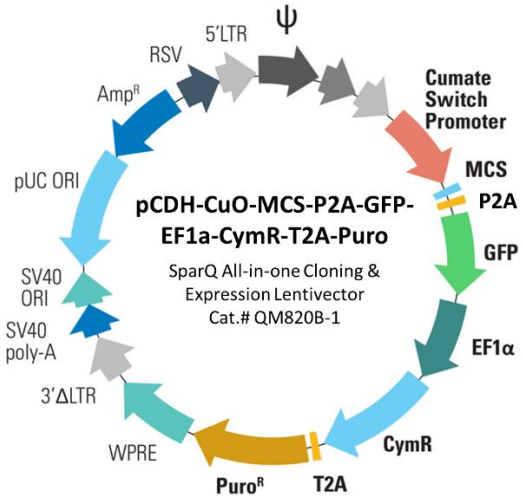
SBI's SparQ™ Cumate Switch System is a gene regulatory system that gives researchers full control of the cells on when and how much a gene-of-interest (protein) can be expressed.

SparQ™2 is adding some of the key advantages over the previous generation and possesses several important elements for researchers to conduct gene modulation projects.

- P2A linker provides higher consistency for reporter expression
- Insert gene size can now be larger than QM812B-1
- Robustness - increase expression up to 40-fold
- Adjustable - tune expression levels by titrating the amount of cumate
- Reversible - turn expression on, off, then on again
- Simple - the all-in-one format allows co-expression of CymR and your gene-of-interest
- Powerful - suitable for *in vivo* applications

Simplify SparQ projects with an all-in-one vector

With SBI's SparQ™2 Cumate Switch System, you can get inducible gene expression in mammalian cells with a range of cloning and expression lentivectors. It simplifies your SparQ™ projects by delivering your gene-of-interest in the same vector that has CymR built-in (vector shown below). The vector drives your gene-of-interest and fluorescent markers (GFP, or RFP) with the inducible cumate switch promoter, and constitutively co-expresses CymR and a resistance marker (puromycin, blasticidin, or neomycin) from an EF1α promoter.



Overall, SparQ™2 system is an excellent choice for achieving highest controlled levels of gene expression. It has negligible background expression in the absence of cumate and is a great approach to study cellular perturbation.

The SparQ™2 cumate switch lentivectors work through virus transduction. Transfection of the vectors will result in constitutive expression from the upstream RSV packaging promoter.

List of Components

Catalog number	Product Name	Quantity
QM820B-1	Plasmid; pCDH-CuO-MCS-P2A-GFP-EF1a-CymR-T2A-Puro	10 µg
QM821B-1	Plasmid; pCDH-CuO-MCS-P2A-RFP-EF1-CymR-T2A-Puro	10 µg
QM822B-1	Plasmid; pCDH-CuO-MCS-P2A-GFP-EF1-CymR-T2A-Blast	10 µg
QM823B-1	Plasmid; pCDH-CuO-MCS-P2A-RFP-EF1-CymR-T2A-Blast	10 µg
QM824B-1	Plasmid; pCDH-CuO-MCS-P2A-GFP-EF1-CymR-T2A-Neo	10 µg
QM825B-1	Plasmid; pCDH-CuO-MCS-P2A-RFP-EF1-CymR-T2A-Neo	10-µg
QM826B-1	Plasmid; pCDH-CuO-RFP-P2A-GFP-EF1-CymR-T2A-Puro	10 µg
QM828B-1	Plasmid; pCDH-CuO-FLuc-P2A-GFP-EF1-CymR-T2A-Puro	10 µg

Storage

The vectors are shipped at room temp, blue ice and dry ice.

Long term storage: at -20°C.

How It Works:

The regulatory mechanisms of the bacterial operons cmt and cym have been engineered to regulate gene expression in mammalian cells. In the repressor configuration, regulation is mediated by strong binding of the CymR repressor to the Cumate operator site (CuO), which is downstream of the CMV5 promoter. Addition of cumate (depicted as yellow circle in the graphic scheme below), a non-toxic small molecule inducer, relieves the repression and drives gene activation; in this case, fluorescent proteins (RFP and GFP). Subsequent removal of cumate from the growth media reverts the activation process, rendering gene switches to off mode.

SBI's SparQ Cumate Switch System can be generalized as follows.

- CymR, a repressor that binds to cumate operator sequences in the absence of cumate
- MCS, to clone-in your gene-of-interest
- Cumate inducible promoter with cumate operator sequences (CuO) upstream of the MCS, to inducibly control gene expression
- Variety of selection markers, to monitor the induction or select stable cell line
- CymR has a high binding affinity for cumate and, as more cumate is added, fewer CymR molecules bind to the CuO sequences in the promoter resulting in increased expression
- Exhibiting much lower background expression than similar systems

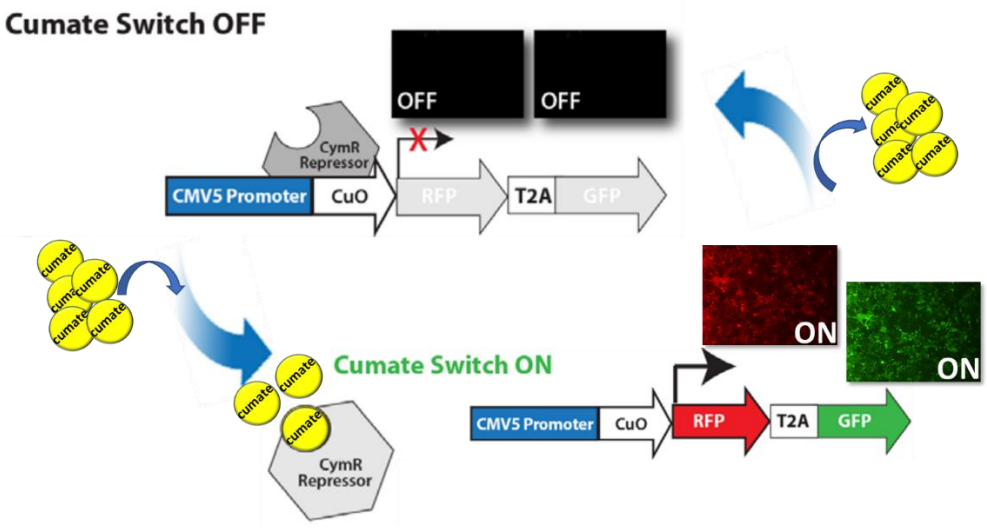


Figure 1. Cumate Switch System

The Workflow At-A-Glance

CHOOSE: Choose a SparQ™ 2 Lentivector with desired reporter and selection marker

CONSTRUCT: Subclone gene of interest into SparQ2 Lentivector

PACKAGE: Package recombinant SparQ™ 2 Constructs into Pseudoviral Particles

TRANSDUCE: Transduction the SparQ™ 2 Viruses into target cells and select with antibiotic.

INDUCE AND MONITOR EXPRESSION: Titrate dose of cumate for desired level of transgene expression.

Protocol for SparQ™2 Cumate Switch System

I. Cloning GOI into SparQTM 2 vector

The following section provides general guidelines for the cloning of cDNA, amplified by PCR, into SparQ™ 2 vectors.

A. cDNA Amplification

Full length cDNA fragments can be recloned from another plasmid or amplified by PCR. The SparQ™ 2 lentivector does not contain an ATG initiation codon. A translation initiation sequence must be incorporated in the insert cDNA if the cDNA fragment to be cloned does not already have an ATG codon. We also recommend including a Kozak sequence (*i.e.* GCCACC) before the ATG for optimal translation. For amplification of the target cDNA fragment, design a 5'-primer (containing a Kozak sequence and ATG codon) and 3'-primer with unique restriction sites present in the MCS of the SparQ™ 2 vector but not present in the cDNA sequence. Since the gene of interest and the reporter gene in SparQ™ 2 vectors containing a P2A peptide sequence, which will form one open-reading frame, extra attention should be paid when designing the 3' primer for amplifying the target sequence. First of all, **do not include a stop codon** at the 3' end of target sequence—this would prevent the expression of the reporter gene; secondly, place the target sequence **in-frame with the 2A peptide**. The ATG start site of the insert that cloned into the MCS must be in-frame with P2A sequence. Please verify the cloning strategy to ensure that this will occur prior to cloning.

Amplify the cDNA fragment by high fidelity DNA polymerase using about 10-50 ng of plasmid template, purify, digest the amplified product with end-specific restriction enzyme(s) and purify the digested PCR product in a 1.2% agarose gel to prevent contamination with the original plasmid used for amplification.

MCS for SparQ™ 2 vector is GCTAGCGAATTCGAATCGCGACCCGGGTGTACA

B. Preparation of Digested SparQ™ 2 Vector

Digest the SparQ™ 2 vector with the corresponding restriction enzymes used in the preparation of the cDNA fragments, and then verify complete digestion of the vector by agarose gel electrophoresis. We suggest that you perform only preparative gel purification of the digested vector if more than one restriction enzyme is used. If you use a single restriction enzyme, dephosphorylation as well as gel purification of the vector is necessary to reduce the background in the vector ligation step.

C. Cloning of cDNA into SparQ™ 2 Vector

1. Cloning of cDNA into SparQ™ 2 Vector

Please refer to the manufacture protocol of the cloning kit you used.

2. Transform *E. coli* with the cloning product

Transform competent cells** (with a transformation efficiency of at least $1x10^9$ colonies/μg pUC19) with the whole ligation reaction (10 μl) following the protocol provided with the competent cells. Plate the transformed bacteria on LB-Ampicillin agar plates.

**Note: We recommend using Stbl2 or OmniMax 2 T1R competent cells for transformation and propagation of the lentivector construct to avoid unwanted lentivector recombination events.

3. Identify Clones with the cDNA Insert

a. Depending on the ratio of colony numbers for the cDNA sample vs. the negative control sample, randomly pick 5 or more well-isolated colonies and grow each clone in 3ml of LB Broth with 75 μg/ml ampicillin at 30°C overnight with shaking.

b. Confirm identity of the cDNA insert by sequence analysis of the clone picked using the PCR primers or the following sequencing primer which is located upstream of the MCS:

Sequencing primer sequence can be used: 5'- CACCTGGCCCGATCTGGCC- 3'

II. Packaging of the SparQ™ 2 Constructs into Pseudoviral Particles

To create a stably transduced cell line, you first need to package the SparQ™ 2 Constructs with GOI into lenti pseudoviral particles. For this purpose, you will need to purchase the pPACKH1 Lentivector Packaging Kit from

SBI. Figure below schematically shows all steps which need to be performed in order to generate pseudoviral packaged SparQ™ 2 Constructs with GOI.

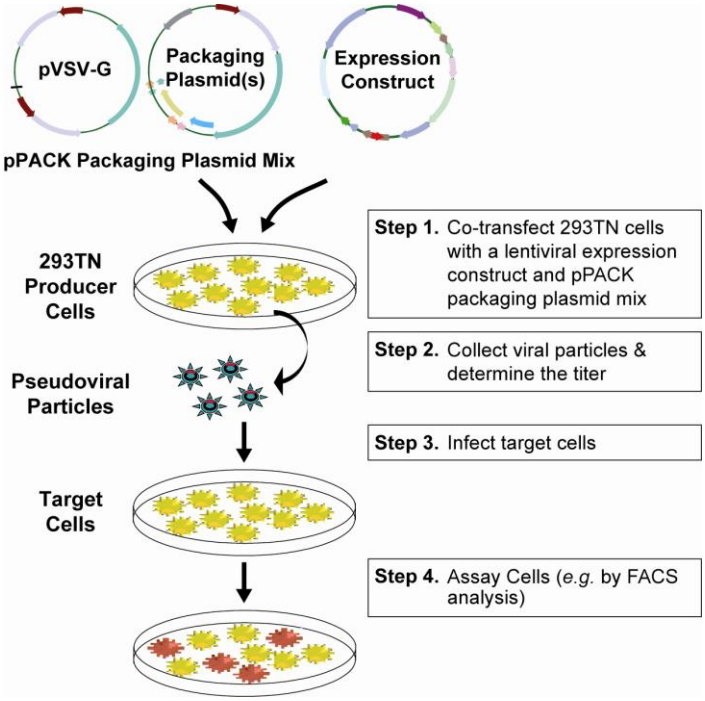


Fig. 2. Schematic presentation of the packaging procedure for lentivector expression constructs and making of stable cell lines.

The Lentivector Expression System User Manual includes the procedural information for packaging and transducing the expression constructs. This user manual is also available on the SBI web site (www.systembio.com).

III. Transduction the SparQTM 2 Viruses

Transduce the SparQ™ 2 Viruses with GOI into your target cells to establish a stable cell line.

Day 1

Plate target cells:

In each well of a 12-well plate, plate your target cells with 1 ml complete growth medium containing S/P so that the cells will be 10 – 30% confluent at the time of infection. Incubate the cells at 37°C with 5% CO_2 overnight.

Day 2

1. Add Transduction reagent: To each well, add TransDux at a final concentration of 1X.
2. Infect target cells with SparQ™ 2 Viruses with GOI
3. In the 12-well plate, add 20, 40 or 80 MOI of each of the packaged viruses. Three days after infection, use 0.5-5 µg/ml puromycin to select for stable cell line, depending upon your cell type.
4. Incubate the cells at 37°C with 5% CO_2.

IV. Induction and Monitoring expression

1. **Induction:** after generate the stable cell line, add Cumate at a final concentration of 1X or desired induction concentration, directly to the wells in which you want to induce the expression of GOI. Continue incubating the cells at 37°C with 5% CO_2. Cumate is supplied as a 1000x solution in 95% ethanol. An easy way to dilute the cumate solution is to make a 100X cumate solution in the cell media you plan to use for your experiment. A 1X solution of cumate corresponds to 30µg/ml. We have tested cumate concentrations between 0.2X and 5X with no toxicity observed in the HEK 293 cells.
2. **Monitor Induction**: When the cumate switch is turned ON, the level of RFP or GFP expression correlates with the induced expression of your GOI cloned into the SparQ™2 construct. Begin monitoring induction after 24 hours, in most cases, easily detectable levels typically occur after 2-3 days.
3. **Turning OFF the cumate switch**: The cumate switch can be turned OFF at any time. Simply remove and change the media of the cells and add fresh media excluding the cumate. The induced expression will gradually fade within 24-72 hours.
4. **Turning the cumate switch back ON**: The cumate switch can be turned back on to test for repeated expression of your gene of interest. Simply add cumate back to the cell media whenever you want to induce the expression again.

Troubleshooting

Cannot obtain Cumate all-in-one stable cell line after antibiotic treatment.

1) The amount of viruses used for infection is too low:
Increase the MOI of the viruses for infection.

2) Optimize the antibiotic centration:

The effective antibiotic working concentration for a target cell line can be determined by establishing a kill-curve on uninfected cells. The concentration of antibiotic that kills >90% of cells after 48h of selection is the correct dose for the cells being selected. In our study, 1μg/ml puromycin was used for selecting stable cells in HEK 293 cells.

Related Products

Cloning of gRNA for Target Gene

Product	Cat#	Website links
pPACKH1 HIV Lentivector Packaging Kit	LV500A-1, LV510A-1	https://www.systembio.com/ppackh1-hiv-lentivector-packaging-kit
PEG-it Virus Precipitation Solution	LV810A-1, LV825A-1	https://www.systembio.com/peg-it-virus-precipitation-solution
TransDux virus transduction reagent	LV850A-1	https://www.systembio.com/transdux-original-virus-transduction-reagent
UltraRapid Lentiviral Global Titering Kit (Human and Mouse compatible)	LV961A-1	https://www.systembio.com/global-ultrarapid-lentiviral-titering-kit

Example Data and Applications

Gene expression with the SparQ™2 Cumate Switch On/Off System

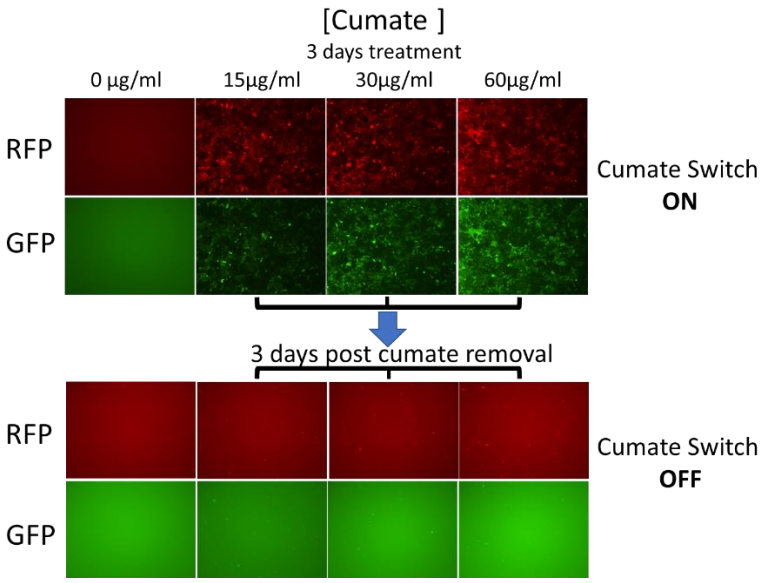


Figure 3. **QM826B-1 was packaged into virus and infected HEK293 cells at MOI of 15. 3 days after infection, 1ug/ml puromycin was added into culture medium to establish stable cell line. Then QM826B-1 HEK293 stable cell line was treated with cumate at difference concentration. 3 days after cumate treatment, RFP and GFP expression was well induced. Cumate was then removed from the culture medium. After 3 days of cumate-free, RFP and GFP expression was barely noticeable.**

Gene expression with the SparQ™2 Cumate Switch System is titratable and reversible.

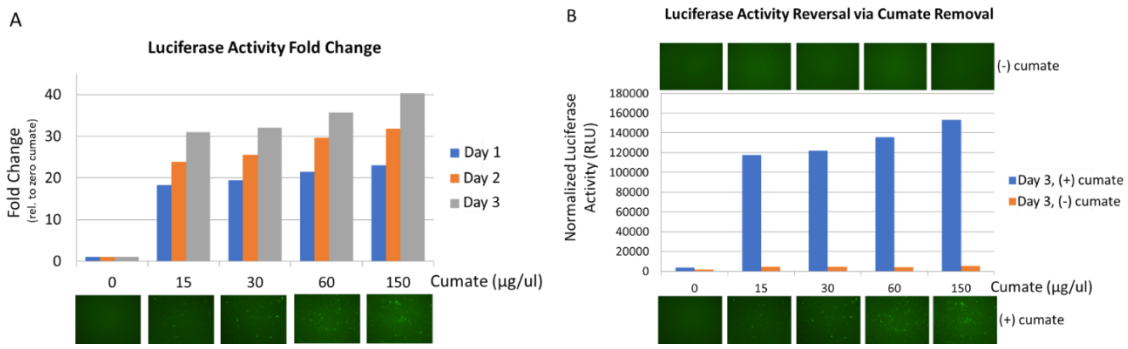


Figure 4. We used QM828B-1 packaged into virus and infected HEK293 cells at MOI of 15. 3 days after infection, 1µg/ml puromycin was added into culture medium to establish stable cell line. (A) QM828B-1 HEK293 stable cell line was treated with cumate at different concentration. Cells were collected at day 1, day 2, and day 3 for luciferase assay to check the luciferase activity. SparQ Cumate Switch System can increase expression up to 40-fold after 3 days of cumate treatment. (B) To inactivate the gene transcription, cumate was removed from the culture medium. After 3 days without cumate treatment, Luciferase and GFP expressed was barely detected.

Gene expression with the SparQ™2 Cumate Switch System is reversible and repeatable

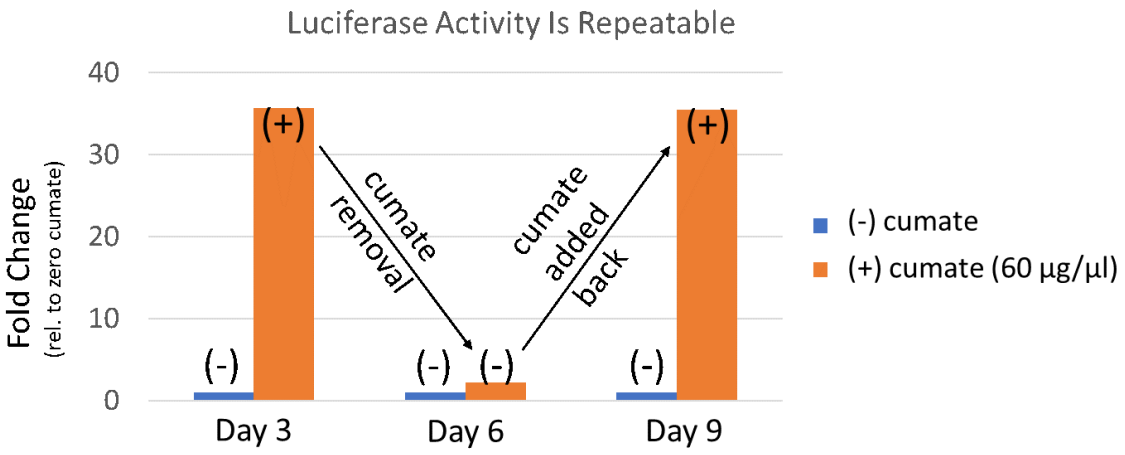


Figure 5. QM828B-1 was packaged into virus and infected HEK293 cells at MOI of 15. 3 days after infection, 1µg/ml puromycin was added into culture medium to establish stable cell line. Then QM828B-1/HEK293 stable cell line was treated with cumate at 60µg/ul. 3 days after cumate treatment, luciferase expression was well induced. Then cumate was removed from the culture medium. After 3 days without cumate treatment, luciferase activity returned to the control level. Finally, when cumate was added back to the culture media, luciferase activity was once again induced.

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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Technical Support: tech@systembio.com
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HIV Vector System

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CMV Promoter

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

CopGFP Reporter

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The Cumate Switch technology

The product contains technologies licensed from the National Research Council, Canada. The technology is covered by the following Patents and Patent Applications.

Country	Type	Number	Date
Canada	Application	2,446,110	5/1/2002
United States	Patent	7,745,592	5/1/2001
United States Continuation	Application	12/769,048	5/1/2001
Belgium	Patent	1385946	5/1/2002
Switzerland	Patent	1385946	5/1/2002
Germany	Patent	1385946	5/1/2002
France	Patent	1385946	5/1/2002
Britain	Patent	1385946	5/1/2002
Ireland	Patent	1385946	5/1/2002
Italy	Patent	1385946	5/1/2002

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