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# AAVS1 SparQ™ All-In-One Inducible System

Cat # GEQM8XXA-1 or KIT

## User Manual

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**Storage:**

Store all reagents at -20°C

Version 1  
9/25/2023

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.



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## Contents

Product Description.....	2
List of Components.....	4
Additional Required and Optional Equipment Not Included in Kit .....	4
Protocol .....	7
A. General Comments.....	7
B. Cloning into AAVS1 SparQ All-In-One inducible donor vectors.....	7
C. Co-transfection of AAVS1 gRNA/Cas9 vector and HR donor plasmids.....	9
D. Single cell sorting for RFP+ and GFP- cell .....	11
E. Characterization of AAVS1 targeted recombinant cells .....	11
F. Induction and Monitoring gene expression.....	13
Supporting Data.....	14
Technical Support .....	16
Licensing and Warranty Statement .....	17



# Product Description

## Controllable Gene Modulation at a Specific Site

Introducing the AAVS1 SparQ™ All-In-One Inducible System, a groundbreaking solution that combines the power of the AAVS1 safe harbor site and CRISPR/Cas9 technology to revolutionize inducible gene expression. This innovative product provides researchers with a comprehensive tool for precise control and manipulation of gene expression, enabling advanced gene therapies, functional genomics studies, and molecular investigations.

Discover the advantages of SBI's AAVS1 SparQ™ All-In-One Inducible System:

- Targeted Integration at AAVS1 Safe Harbor Site
- Inducible Gene Expression
- High Specificity
- Effortless Cell Line Generation
- Easy Use with All-In-One Design

## Streamline Inducible Expression Cell Line Generation from the Powerful AAVS1 Site

The AAVS1 safe harbor site, located on chromosome 19 in humans, offers a reliable and stable genomic locus for targeted gene integration. The SparQ™ All-In-One Inducible Expression Cassette utilizes a small molecule inducer, cumate, to achieve precise control over gene expression in high efficiency. Combined with the versatility of CRISPR/Cas9 technology, the AAVS1 SparQ™ All-In-One Inducible System provides a robust platform for controlled transgene expression and manipulation within AAVS1 safe harbor site.

To streamline your research journey, our AAVS1 SparQ™ All-In-One Donor Vectors come with AAVS1 homology arms pre-cloned. Your task? Simply introduce your gene of interest and co-transfect with a Cas9/AAVS1 gRNA complex, such as our top-tier All-in-one Cas9 SmartNuclease & AAVS1 gRNA Plasmid

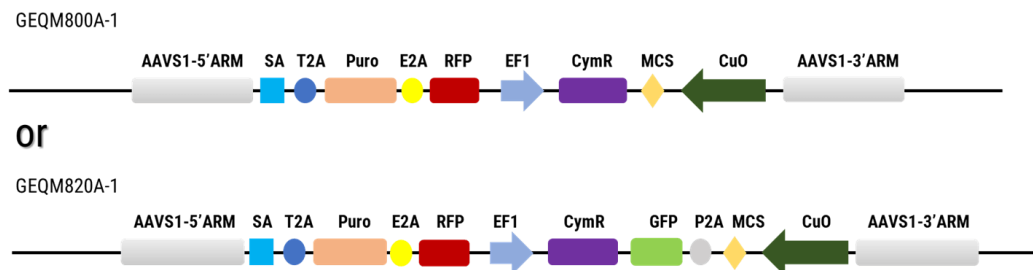


Figure 1. AAVS1 SparQ™ All-In-One Donor Vectors.

### **SBI's AAVS1 SparQ™ All-In-One Inducible System Offers:**

**Targeted Integration at AAVS1 Safe Harbor Site:** Harness the precision of CRISPR/Cas9 technology to achieve seamlessly integration at the AAVS1 safe harbor site for reliable gene control.

**Inducible Gene Expression:** Achieve precise control over gene expression from AAVS1 safe harbor site, allowing you to turn genes on or off at will.

**High Specificity:** Experience minimal off target integration

**Effortless Cell Line Generation:** Streamline the construction of site-specific inducible isogenic cell lines.

**Easy Use with All-In-One Design:** A unprecedented solution that simplifies the entire process of inducible gene expression, from targeted integration to control.

### **Why AAVS1?**

The AAVS1 safe harbor site stands out as a prime target for gene knock-ins, consistently delivering potent transgene expression. Research has demonstrated that insertions at this site are not only safe but also free from any reported phenotypic effects. Moreover, the neighboring DNA remains in an open conformation, ensuring dependable expression across a range of transgenes. With the integration of CRISPR/Cas9 technology, the AAVS1 SparQ™ All-In-One Inducible System facilitates precise embedding of the cumate-inducible expression cassette right at the AAVS1 site. Such strategic placement ensures minimal interference with adjacent genes, presenting a holistic solution for meticulous gene manipulation and regulated expression.

### **Why Choose One of Our AAVS1 SparQ™ All-In-One Donor Vectors?**

Our AAVS1 SparQ™ All-In-One Donor Vectors are ingeniously crafted to ensure minimal off-target integration for highly-specific targeting at the AAVS1 site. Leveraging the AAVS1's strategic position within an intron, the puromycin marker is equipped solely with a splice acceptor site without a promoter. This ensures that puromycin expression is activated only upon intron integration, significantly reducing the chances of off-target integrants during puromycin selection.

The All-In-One design incorporates all essential elements required for inducible gene expression into a single, compact construct. It includes the regulatory elements, inducible Cumate Switch promoter (CuO), multiple cloning sites for transgene or miRNA and the cumate repressor (CymR) for inducible control. This streamlined design simplifies experimental workflows, saves time, and enhances research efficiency.

By combining the power of the AAVS1 safe harbor site and CRISPR/Cas9 technology, the AAVS1 SparQ™ All-In-One Inducible System empowers researchers to unlock the full potential of inducible gene expression and precise gene editing. Explore new frontiers in gene therapy, functional genomics, and molecular research with this comprehensive solution and drive scientific advancements with unparalleled control and precision.

## List of Components

**Table 1. Components of GEQM8XXA-1 or KIT products**

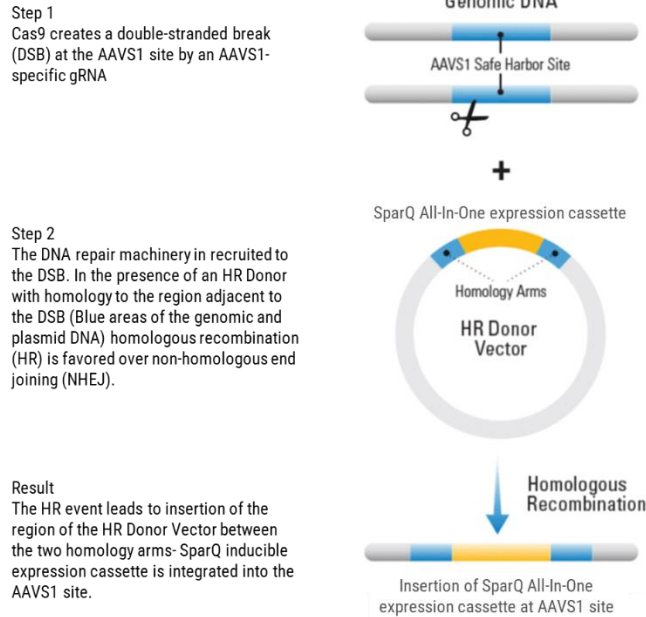
Catalog Number	Product Name	Quantity
GEQM800A-1	AAVS1 SparQ™ All-In-One Inducible Donor Vector (AAVS1-SA-RFP-Puro-EF1-CymR-CuO-MCS)	10 µg
GEQM820A-1	AAVS1 SparQ™ All-In-One Inducible Donor Vector (AAVS1-SA-RFP-Puro-EF1-CymR-CuO-MCS-P2A-GFP)	10 µg
GEQM800A-KIT	AAVS1 SparQ™ All-In-One Inducible System includes GEQM800A-1 (AAVS1-SA-RFP-Puro-EF1-CymR-CuO-MCS) with CAS601A-1 (All-in-one Cas9 SmartNuclease AAVS1 Targeting Plasmid) and GEQM840PR-1 (Junction PCR Primer Mix to confirm AAVS1 site integration)	1 Kit
GEQM820A-KIT	AAVS1 SparQ™ All-In-One Inducible System GEQM820A-1(AAVS1-SA-RFP-Puro-EF1-CymR-CuO-MCS-P2A-GFP) with CAS601A-1 (All-in-one Cas9 SmartNuclease AAVS1 Targeting Plasmid) and GEQM840PR-1 (Junction PCR Primer Mix to confirm AAVS1 site integration)	1 Kit
GEQM840PR-1	5' and 3' Primer Mixes for Junction PCR Assays for GEQM-8xx (AAVS1 SparQ™ All-In-One Inducible System, 10 µM)	100 µl

## Additional Required and Optional Equipment Not Included in Kit

1. Plasmid propagation: RecA- and EndA- E.coli competent cell (We use OneShot OmniMax 2T1R in house), Kanamycin, Carbenecillin
2. Sub cloning: HF restriction enzymes, agarose gel, gel clean up kit, Cold Fusion Cloning Kit or ligase
3. Cas9/AAVS1 targeting plasmid: CAS601A-1
4. Selection: Puromycin, FACs instrument
5. Validation: Polymerase, PCR Cycler, may need GEQM840A-1
6. Cumate for Induction: QM100A-1 or QM150A-1

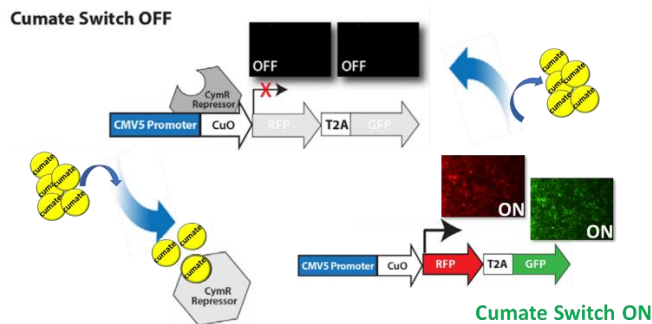
# How it works

## SparQ™ All-in-One Expression Cassette Knock-In at AAVS1 site



**Figure 2. Knocking-in SparQ™ All-In-One Expression Cassette at the AAVS1 Site. Step 1: Cas9 introduces a double-stranded break (DSB) at the AAVS1 site. The activity of Cas9 is directed to the AAVS1 site by an AAVS1-specific gRNA. Step 2: The DNA repair machinery is summoned to the DSB. When an HR Donor, which has homology to the region adjacent to the DSB (represented by the blue areas on both the genomic and plasmid DNA), is present, homologous recombination (HR) takes precedence over non-homologous end joining (NHEJ). Result: The HR event results in the insertion of the region from the HR Donor Vector between the two homology arms. As a consequence, the SparQ™ All-In-One expression cassette becomes integrated into the AAVS1 site.**

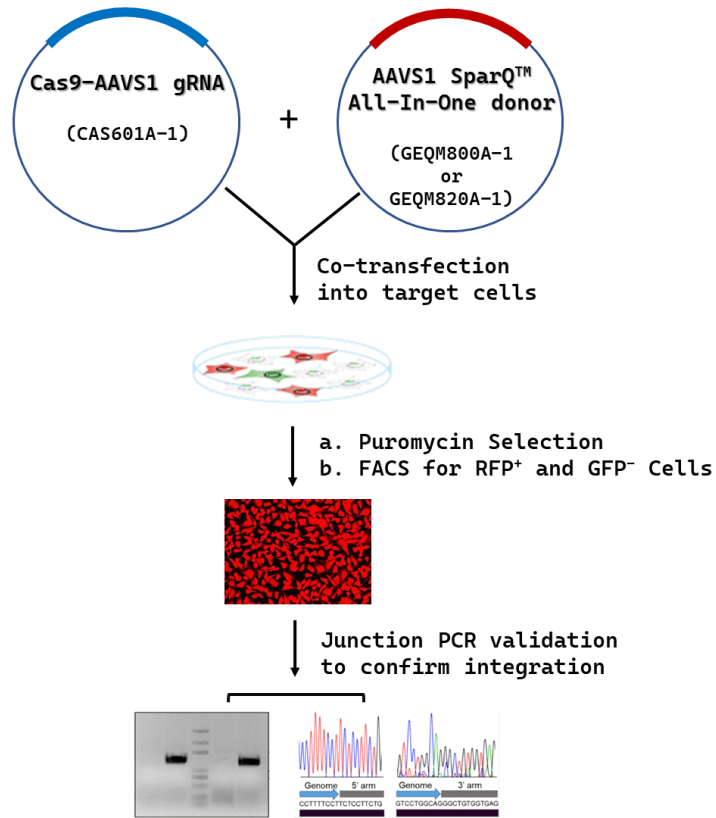
## SparQ™ Cumate Switch System:



**Figure 3. SparQ™ Cumate Switch System. The regulatory mechanisms of the bacterial operons *cmt* and *cym* have been engineered to regulate gene expression in mammalian cells. In the repressed 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 to switch to off mode.**



## Workflow for Generation of Site Specific Inducible Isogenic Cell Line:



**Figure 4. Generating an inducible expression cell line using the AAVS1 SparQ™ All-In-One System. Step 1: Co-transfect your target cell line with the all-in-one vector, which expresses both Cas9 and AAVS1-specific gRNA, along with the AAVS1 SparQ™ All-in-One donor vector GEQM800A-1 or GEQM820A-1 containing your transgene or miRNA. Step 2: Four to seven days after transfection, apply puromycin for stable cell line selection. Step 3: Perform single-cell sorting using FACS to isolate RFP positive (RFP<sup>+</sup>) and GFP negative (GFP<sup>-</sup>) cells. Step 4: For the clonal cell line, validate precise integration at the AAVS1 site using junction PCR. Step 5: Manipulate gene expression using cumate.**

# Protocol

## A. General Comments

We recommend propagation of the plasmids provided in the AAVS1 SparQ™ All-In-One inducible system prior to starting the experiments. The plasmids can be transformed using standard conditions suitable in any RecA- and EndA- E.coli competent cell.

Cells with the AAVS1 SparQ™ All-In-One inducible donor vectors should be grown on LB-Carbenicillin plates (50µg/ml). Cells with the AAVS1 gRNA/Cas9 targeting vectors should be grown on LB-Kanamycin plates (50µg/ml). Incubate the plates at 37°C overnight. Colonies picked from the transformation can be grown at 37°C overnight in ~200ml of LB media containing carbenicillin or kanamycin, respectively. After overnight growth, plasmid DNA can be harvested from culture using an endotoxin-free DNA plasmid maxiprep kit.

For confirmation of the plasmid, we recommend performing restriction digestion analysis or direct sequencing to confirm integrity of the amplified plasmids.

## B. Cloning into AAVS1 SparQ™ All-In-One inducible donor vectors

For rapid and efficient cloning of any insert into the donor vector, we recommend SBI's **Cold Fusion Cloning Kit** as a ligase and restriction enzyme-free cloning method. More details can be found here:

<http://www.systembio.com/molecular-tools/cold-fusion-cloning/overview>

The following section provides general guidelines for the cloning of cDNA, amplified by PCR, into AAVS1 SparQ™ All-In-One inducible donor vectors.

### 1. cDNA Amplification

Full length cDNA fragments can be recloned from another plasmid or amplified by PCR. The AAVS1 SparQ™ All-In-One inducible donor vectors do 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 with unique restriction enzyme-Kozak sequence-ATG followed by

specific sequence of GOI and 3'-primer with 3'end specific sequence of GOI-Stop codon-unique restriction sites present in the MCS of the AAVS1 SparQ™ All-In-One inducible donor vector but not present in the cDNA sequence.

**Please note** that since the gene of interest and the reporter gene in GEQM820A-1 is linked by 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 with the same orientation of CuO promoter for AAVS1 SparQ™ All-In-One inducible donor vectors is:**

**5' TGTACATTCGAAGATATCGGTACCTCGCGA 3'**

**Please pay attention to** the orientation of CuO promoter and make sure the ORF was in the same orientation as CuO promoter

## **2. Preparation of Linearized AAVS1 SparQ™ All-In-One inducible donor vectors**

Linearize the AAVS1 SparQ™ All-In-One inducible donor 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.

## **3. Ligation of insert into vector**

- a) Dilute gel-purified, digested vector to 10-50ng/μl
- b) Set up 10μl ligation reactions for each control and test samples as below:

<u>Volume</u>	<u>Item</u>
<b>10-100ng</b>	Linearized AAVS1 SparQ All-In-One inducible vector
<b>variable</b>	Insert DNA (at 3:1 molar excess over vector)
<b>2.0 µl</b>	5x Rapid ligation buffer
<b>0.5 µl</b>	T4 DNA Ligase (5 U/µl)
<b>10.0 µl</b>	Total Reaction Volume

- c) Incubate reactions at 22°C for 5 minutes

#### 4. Transform *E. coli* with the ligation product

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

#### 5. Identify clones with the correct insert by enzyme digestion or PCR

6. **Maxiprep the positive clone.** Grow a positive clone containing insert in an appropriate amount of LB-Ampicillin/Carbenicillin Broth, and purify the construct using an endotoxin-free plasmid purification kit. sequence verification of the insert is optional.

### C. Co-transfection of AAVS1 gRNA/Cas9 vector and HR donor plasmids

- 1) Plate ~100,000 cells/well in a 12-well plate according to established recommended conditions for cell type(s) being transfected. Include wells for the following:

- a) AAVS1 gRNA/Cas9 vector + AAVS1 SparQ™ All-In-One inducible donor vector  
b) AAVS1 SparQ™ All-In-One inducible donor vector only

- 2) Next day, prepare transfection complexes of AAVS1 gRNA/Cas9 vector and AAVS1 SparQ™ All-In-One inducible donor plasmids using a suitable transfection reagent such as Lipofectamine 2000 according to the manufacturer's recommended instructions. Leave the transfection complex on the cells for >6 hours.

**Example: For HEK293T cells using Lipofectamine 2000 reagent, transfect 0.5µg of CAS601A-1, AAVS1 gRNA/Cas9 vector and 0.5µg of AAVS1 SparQ™ All-In-One inducible donor vector.**

**Tech Notes:**

- a) Since transfection efficiencies vary across different cell lines, we recommend optimizing the input of AAVS1 gRNA/Cas9 plasmid to HR donor plasmid for best results.
- b) For optimal results, we recommend complexing of DNA with transfection reagent in serum- and antibiotic-free media and cells growing in complete media (e.g. DMEM/F12+10% FBS w/o antibiotics).
- c) For hard-to-transfect cells (e.g. primary, stem, hematopoietic), it may be advisable to utilize a non-passive transfection method such as NucleoFection (Lonza) or Neon system (Life Technologies). Please follow recommended transfection guidelines provided by the manufacturer for specific cell type(s) being transfected.
- 3) 24 hours post-transfection, remove transfection media and transfer the cells in 10 cm dish with complete growth media.
- 4) Begin puromycin selection 4-7 days post-transfection. For 293T cells, the recommended concentration of puromycin is 1 µg/ml.

**Tech Note:**

**The effective working puromycin concentration for a target cell line can be determined by establishing a kill-curve on untransfected cells. The concentration of puromycin (typical working range of 0.5µg-5µg/ml) that kills >90% of cells after 48hours of selection is the correct dose for the cells being selected.**

5) Puromycin selection for about 7-10 days,

a) AAVS1 gRNA/Cas9 vector + AAVS1 SparQ™ All-In-One inducible donor:

Resulting colonies should be Puro resistant.

b) AAVS1 SparQ™ All-In-One inducible donor only

Very few colonies (if any) should be seen relative to AAVS1 SparQ™ All-In-One inducible donor + gRNA/Cas9 vector (Sample a). Presence of Puro<sup>R</sup> colonies indicates frequency of random integration events.

#### **D. Single cell sorting for RFP+ and GFP- cell**

- 1) Single cell sorting for individual cell that is RFP+ and GFP- after puromycin selection with FACS.
- 2) Culture RFP+ and GFP- clonal cells in 96-well plate for 1-2 weeks.

#### **E. Characterization of AAVS1 targeted recombinant cells**

- 1) For confirmation of donor vector integration specifically at the AAVS1 target locus, junction-PCR can be performed using PCR primer pairs that flank the 5' AAVS1 homology arm (5' AAVS1-HA-L) and 3' AAVS1 homology arm (3' AAVS1-HA-R). Junction-PCR primers are provided in the AAVS1 Targeted Integration Kits or sold separately as GEQM840PR-1.
- 2) Donor Vector Junction-PCR
  - a) Primer Sequences

##### 5' junction PCR primers:

5' junction PCR-fwd: TCCTGAGTCCGGACCACTTT

5' junction PCR-rev: CACCGCATGTTAGAAGACTTC

5' junction PCR amplicon: 1004bp

##### 3' junction PCR primers:

3' junction PCR-fwd: GCGGGCCATTTACCGTAAG

3' junction PCR-rev: cacagttggaggagaatccac

3' junction PCR amplicon: 1080bp

The primers are provided as mixes (F/R primers) at 10 $\mu$ M. Validation of either the 5' or 3' homology arms for donor integration is usually sufficient; however, both arms can be done for additional confirmation.

b) Protocol Details for Junction-PCR Assay:

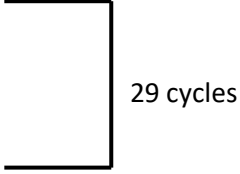
- i. Isolate genomic DNA from positive control cells using a suitable genomic DNA isolation kit for extraction of gDNA from cultured cells. Please follow manufacturer's recommended protocol.
- ii. Perform Junction-PCR (PCR reaction below)

Reagent	AAVS1 gRNA/Cas9 vector + AAVS1 SparQ All-In-One inducible donor	AAVS1 SparQ All-In-One inducible donor only Or parental cell line
5X Phusion GC Buffer	5 $\mu$ l	5 $\mu$ l
10mM dNTPs	0.5 $\mu$ l	0.5 $\mu$ l
10 $\mu$ M 5' junction PCR mix Or 3' junction PCR mix	2.5 $\mu$ l	2.5 $\mu$ l
DMSO	0.75 $\mu$ l	0.75 $\mu$ l
Phusion DNA Polymerase (2 U/ $\mu$ L)	0.25 $\mu$ l	0.25 $\mu$ l
Genomic DNA from cells transfected with AAVS1 gRNA/Cas9 vector + AAVS1 SparQ <sup>TM</sup> All-In-One inducible donor (100ng/ $\mu$ l)	0.3-0.5 $\mu$ l	N/A
Genomic DNA from cells transfected with AAVS1 SparQ <sup>TM</sup> All-In-One inducible donor only or parental cells (100ng/ $\mu$ l)	N/A	0.3-0.5 $\mu$ l
PCR-grade H <sub>2</sub> O	25 $\mu$ l	25 $\mu$ l

iii. Proceed with PCR protocol below:

98°C, 3 min

98°C, 30 sec  
59°C, 15 sec  
72°C, 90 sec



29 cycles

72°C, 2 min

Hold at 4°C

iv. Run the PCR reaction out on a 1.5% agarose/EtBr gel in 1X TBE buffer to confirm the Junction-PCR results.

#### F. Induction and Monitoring gene 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<sub>2</sub>. 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 GFP expression correlates with the induced expression of your GOI cloned into the GEQM820A-1. Begin monitoring induction after 24 hours, in most cases, easily detectable levels typically occur after 2 days.

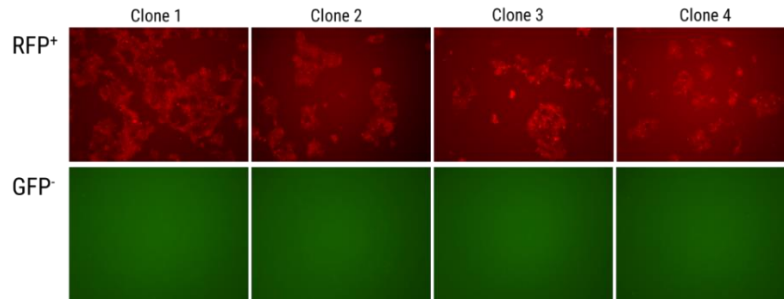
3. Turning OFF the cumate switch: The cumate switch can be turned OFF at any time. Simply remove and change the media of the cells and add fresh media excluding the cumate. The induced expression will gradually fade within 48-72 hours.

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.



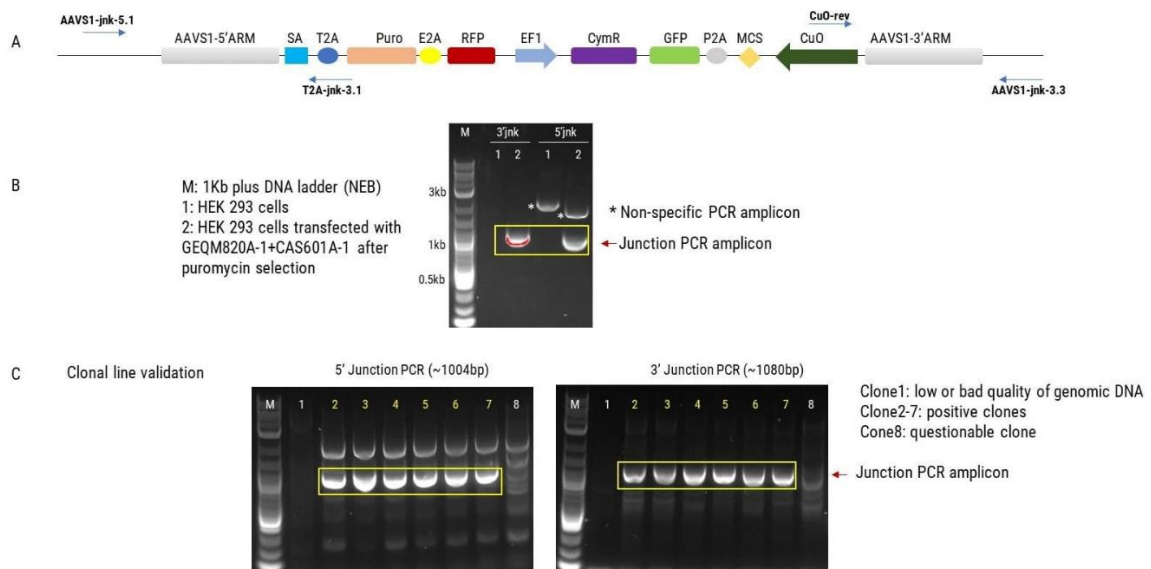
## Supporting Data

### AAVS1 SparQ™ Inducible Cell Line is RFP<sup>+</sup> and GFP<sup>-</sup> before Induction



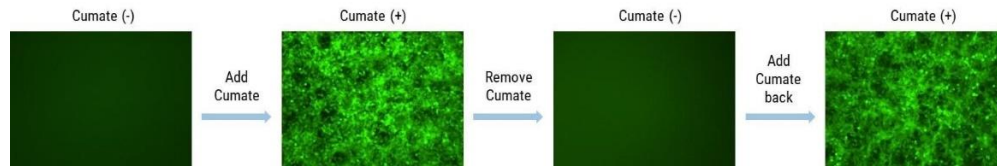
**Figure 5.** Selected clonal cell lines derived from AAVS1 SparQ™ inducible system. The HEK 293 cells were co-transfected with GEQM820A-1 and CAS601A-1. After puromycin selection (1 µg/ml), single-cell sorting was performed using FACS to isolate RFP<sup>+</sup> and GFP<sup>-</sup> cells.

### Junction PCR Validation for Precise Integration of the SparQ™ All-in-One Expression Cassette at the AAVS1 Site



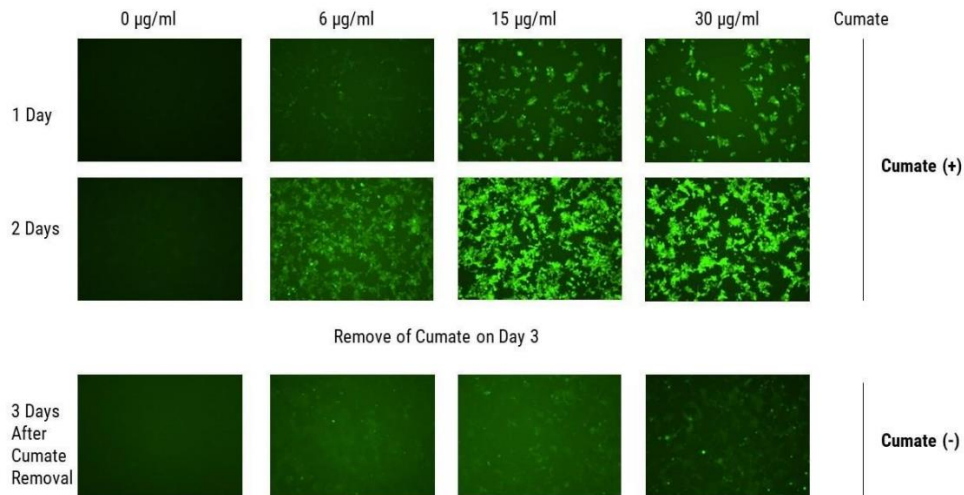
**Figure 6.** The AAVS1 SparQ™ All-in-One expression cell line is specifically integrated at the AAVS1 site. The HEK 293 cells were co-transfected with GEQM820A-1 and CAS601A-1. Following puromycin selection (1 µg/ml) and single-cell sorting using FACS for RFP<sup>+</sup> and GFP<sup>-</sup> cells, individual clonal cell lines were generated. Junction PCR was used to verify the precise integration of the SparQ™ All-in-One expression cassette at the AAVS1 site. A) Illustration of the junction PCR design. B) Junction PCR validation using pooled puromycin-resistant cells. C) Validation of individual clonal cell lines by junction PCR.

## Gene Expression is Inducible and Reversible



**Figure 7.** Gene expression in the AAVS1 SparQ™ All-in-One expression cell line is inducible and reversible. GFP expression in the GEQM820A-1 stable cell line was induced just one day after adding cumate (cumate (+)) at 30 µg/ml; images were taken on day 2 post-cumate addition. Upon removal of cumate (cumate (-)) from the culture medium, GFP expression gradually diminished. Reintroducing cumate to the culture medium led to further induction of GFP expression.

## Precise Control Over Expression Dynamics



**Figure 8.** Achieve fine-tuned gene regulation in the AAVS1 SparQ™ All-in-One expression cell line. The SparQ™ inducible expression cassette allows for tight and dose-dependent control over transgene expressions. GFP expression in the GEQM820A-1 stable cell line was induced by adding cumate at various dosages. Images were taken on 1 day and 2 days after adding cumate. On day 3, cumate was removed from the culture medium, and GFP expression gradually diminished accordingly. Images were taken on 3 days after removing of cumate. Researchers can modulate gene expression levels simply by adjusting the concentration of cumate, enabling precise control over expression dynamics for their specific research needs.

## 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:

System Biosciences (SBI)  
2438 Embarcadero Way  
Palo Alto, CA 94303

**Phone:** (650) 968-2200

**Toll-Free:** (888) 266-5066

**Fax** (650) 968-2277

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Technical Support: [tech@systembio.com](mailto:tech@systembio.com)

Ordering Information: [orders@systembio.com](mailto:orders@systembio.com)

## Licensing and Warranty Statement

### Acknowledgement:

Design of the pZT-AAVS1-L1/R1 and pAAVS1D-CMV-RFP-EF1 $\alpha$ -copGFP-T2A-Puro vectors was performed by Dr. Jizhong Zou of the NIH Center for Regenerative Medicine, a Common Fund initiative of the U.S. National Institutes of Health.

### Limited Use License

Use of the AAVS1 SparQ™ All-In-One Inducible System (*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.

This Product is covered by licensed Patents. 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.

### *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

SBI has pending patent applications on various features and components of 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.



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