CRISPR-Cas9-AAVS1 Safe Harbor Site Targeting System Overview

Highlights:

- Validated and well-characterized
- Straightforward and easy workflow
- Precise integration of any gene at AAVS1 safe harbor site
- Distinctive design ensures robust and consistent transgene expression
- Minimal off-target integration risk
- Efficient process for CRISPR/Cas9 library screening
- A versatile combination approach provides the most extensive options for CRISPR/Cas9-AAVS1 Safe Harbor Site Targeting System

One of the most powerful applications of genome editing with the CRISPR/Cas9 system is the introduction of precise modifications at a targeted location, which exploits the homology-directed repair (HDR) pathway in mammalian cells. An important application involves knocking in an expression cassette into the AAVS1 safe-harbor site (PPP1R12C). This technique allows the introduction of a transgene for overexpression and rescue studies without disrupting the expression of endogenous genes. Such studies not only provide insights into disease mechanisms but also offer potential therapeutic treatments for various types of disease.

ALSTEM has developed its CRISPR/Cas9-based AAVS1 Safe Harbor Site Targeting System for safe and easy gene knock-in. We offer an array of tools designed to help researchers accelerating their research by leveraging the AAVS1 safe harbor site.

Using ALSTEM CRISPR/Cas9 platform as a foundation, we designed an all-in-one vector containing the spCas9 nuclease and a gRNA targeting the AAVS1 safe harbor site. These vectors provide strong expression of the spCas9 protein and a specific guide RNA, ensuring precise cleavage at the AAVS1 safe harbor site with minimal off-target cutting.

- pSpCas9 nuclease & AAVS1 gRNA Vector, Cat#GA001

Streamline of the process of generating stable cell lines with options including multiple selection markers, reporter lines, any desired gene, inducible expression, tissue-specific expression, and additional features.

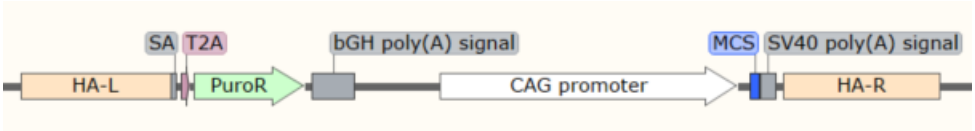
Utilizing our latest AAVS1 donor vectors, you gain diverse avenues for seamlessly integrating your gene of interest into the AAVS1 safe harbor locus via the CRISPR/Cas9 platform. For constant

ALSTEM, INC
2600 Hilltop Drive, B/C328
Richmond, CA 94806 USA

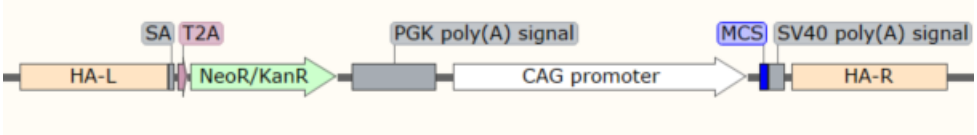
+1 (510) 708-0096
info@alstembio.com
www.alstembio.com

expression of any desired gene, we offer our second-generation vectors with a multiple cloning site (MCS) strategically located downstream of a CAG promoter and upstream of a poly-A tail. Additionally, for a more convenient method of generating your stable cell line, we offer advanced options with multiple selection markers including Hygromycin, Neomycin, and Puromycin. The expression of the SA-T2A-selection marker is intricately associated with the PPP1R12C transcript, which corresponds to the integration of your gene of interest into the AAVS1 safe harbor site.

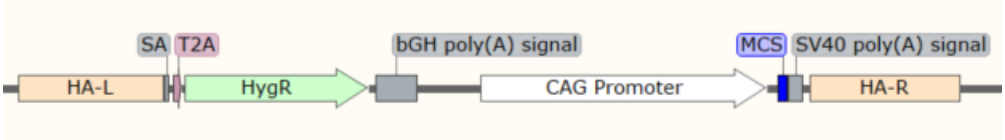
- pAAVS1-SA-T2A-Puro-CAG-MCS-SV40pA, Cat#AC2301



- pAAVS1-SA-T2A-Neo- CAG-MCS-SV40pA, Cat#AC2302

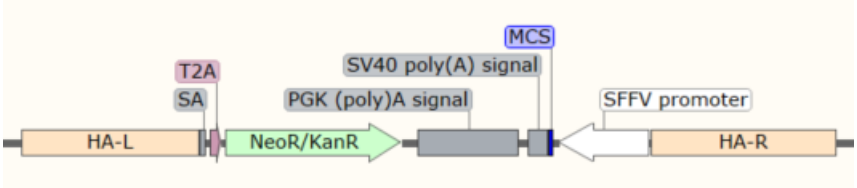


- pAAVS1-SA-T2A-HygR-CAG-MCS-SV40pA, Cat#AC2303



To achieve a more robust and powerful expression of the Gene of Interest (GOI) while minimizing interference with PPP1R12C transcripts, we provide an additional option: a reverse orientation expression cassette utilizing the SFFV promoter.

- pAAVS1-SA-T2A-Neo-SV40pA-MCS-SFFV, Cat#AC2402



Alternatively, if you wish to insert a combination of a promoter and gene, we provide an identical donor vector without a promoter and poly-A; instead, it features only an MCS located upstream of HA-R.

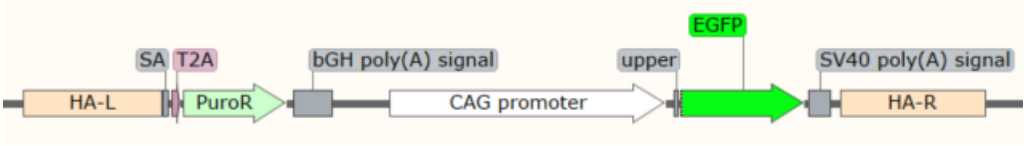
ALSTEM, INC
2600 Hilltop Drive, B/C328
Richmond, CA 94806 USA

+1 (510) 708-0096
info@alstembio.com
www.alstembio.com

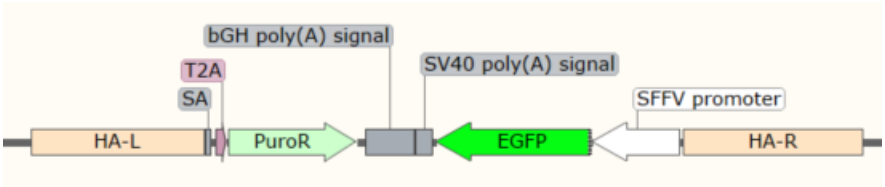
- pAAVS1-SA-T2A-Puro-SV40pA-MCS, CAT#AC2002

For your ease and convenience, we provide transfection-positive control donor vectors equipped with a variety of selectable markers driven by a CAG promoter or an SFFV promoter in reverse orientation.

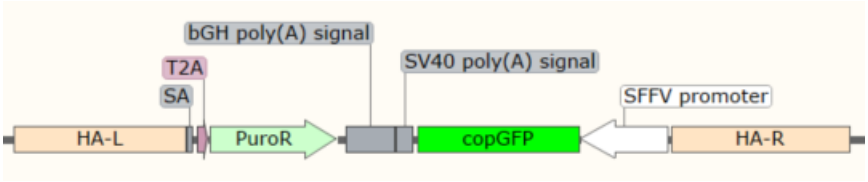
- pAAVS1-SA-T2A-Puro-CAG-EGFP-SV40pA, Cat#AC9311



- pAAVS1-SA-T2A-Puro-SV40pA-EGFP-SFFV, Cat#AC9411



- pAAVS1-SA-T2A-Puro-SV40pA-copGFP-SFFV, Cat#AC9421



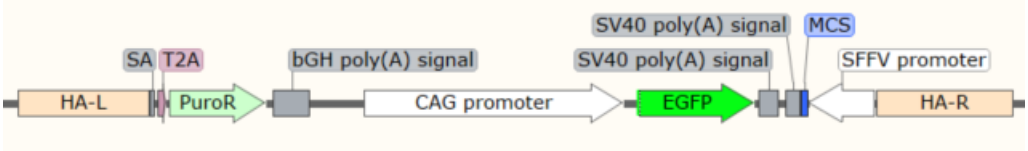
- pAAVS1-SA-T2A-Neo-CAG-EGFP-SV40pA, Cat#AC9312

We've streamlined the process of creating reporter cell lines using an AAVS1 donor vector. It contains a CAG-driven EGFP combined with an MCS under a robust and stable SFFV promoter in reverse orientation, followed by a downstream SV40 poly-A tail.

ALSTEM, INC
2600 Hilltop Drive, B/C328
Richmond, CA 94806 USA

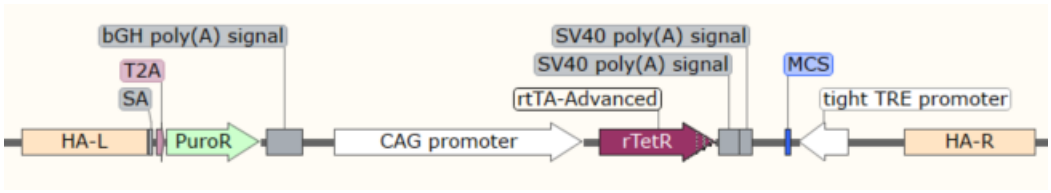
+1 (510) 708-0096
info@alstembio.com
www.alstembio.com

- pAAVS1-SA-T2A-Puro-CAG-EGFP-SV40pA-SV40pA-MCS-SFFV, Cat#AC4411

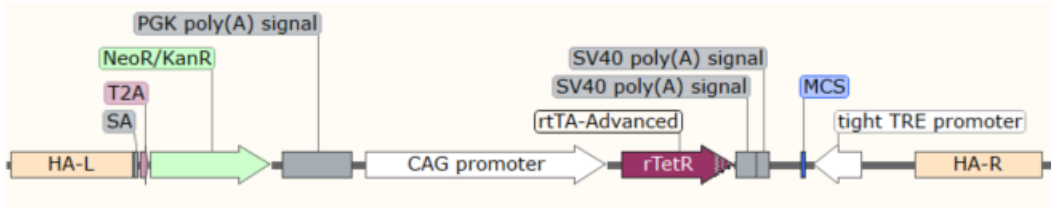


Further key development resulted a highly flexible all-in-one inducible AAVS1 donor vector that is equipped with a well-established TetOn regulatory element. We offer this with multiple selection markers, such as Neomycin, Puromycin and Hygromycin, to accommodate various gene-of-interest (GOI) designs. For example, you can simultaneously integrate two distinct GOIs into a cell line by incorporating your GOIs into different alleles.

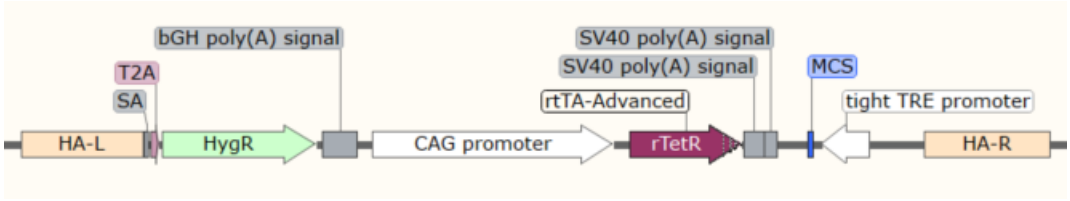
- pAAVS1-SA-T2A-PuroR-CAG-rtTA-SV40pA-TRE-MCS-SV40pA, Cat#3901



- pAAVS1-SA-T2A-NeoR-CAG-rtTA-SV40pA-TRE-MCS-SV40pA, Cat#AI3902



- pAAVS1-SA-T2A-HygR-CAG-rtTA-SV40pA-TRE-MCS-SV40pA, Cat#AI3903

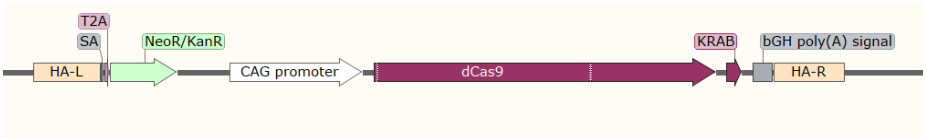


Lastly, ALSTEM also provides a CRISPRi gene silencing vector based on dCas9 with dCas9/KRAB, along with a CRISPRa gene activation vector, dCas9/VPR. For inducible control of gene silencing and activation, we offer an all-in-one AAVS1 donor vector equipped with a well-established TetOn regulatory element.

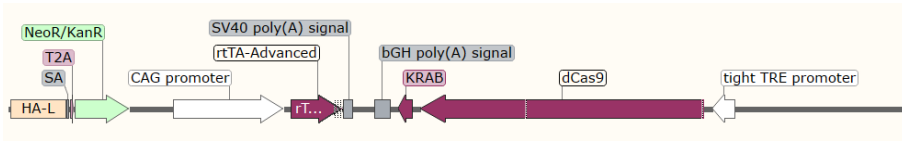
ALSTEM, INC
2600 Hilltop Drive, B/C328
Richmond, CA 94806 USA

+1 (510) 708-0096
info@alstembio.com
www.alstembio.com

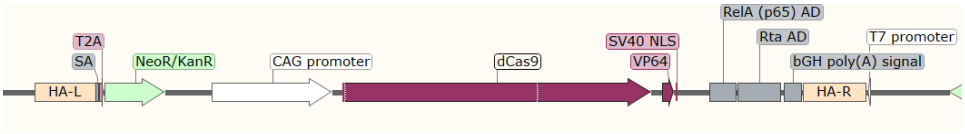
- pAAVS1-SA-T2A-Neo-CAG-dCas9-KRAB-pA Donor Vector CAT#AC7302



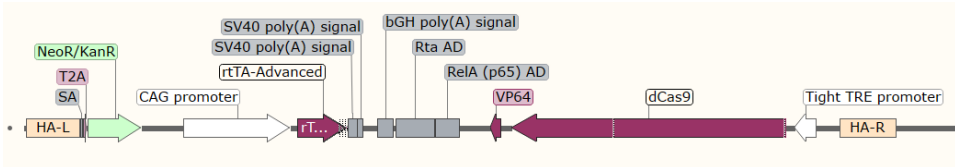
- pAAVS1-SA-T2A-Neo-CAG-rtTA-pA-TRE-dCas9-KRAB-pA Donor Vector CAT#AI7902



- pAAVS1-SA-T2A-Neo-CAG-dCas9-VPR-bGHpA CAT#AC8302



- pAAVS1-SA-T2A-Neo-CAG-rtTA-SV40pA-TRE-dCas9-VPR-bGHpA CAT#AI8902



ALSTEM, INC
2600 Hilltop Drive, B/C328
Richmond, CA 94806 USA

+1 (510) 708-0096
info@alstembio.com
www.alstembio.com