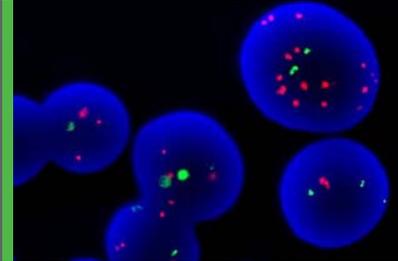


Enhanced Episomal Vectors (EEV)

Sustained, non-integrating transgene expression



The EEV system avoids the random integration and mutagenesis challenges of viral transduction systems. The EEV technology is based on the Epstein-Barr Nuclear Antigen-1 (oriP-EBNA1) and the major distinguishing feature of the EBNA system from viral or other plasmid-based approaches is its ability to replicate in synchrony with the host genome by attaching to the host chromatin and replicate with each cell cycle division. This results in an extended presence within a host cell. The target gene to be expressed (such as a reporter or iPSC reprogramming factors) can be expressed in the same plasmid as the oriP-EBNA1 factor for sustained transgene expression. Over time, these episomal plasmids are naturally lost at a rate of 5% per cell cycle division due to plasmid dilution, promoter silencing, and vector replication errors. Since most of the plasmid is lost over time, cell lines can then be established without any risk of genomic integration or alterations. SBI has developed an enhanced version of the oriP-EBNA1 technology called the Enhanced Episomal Vector (EEV) platform. This new technology enables sustained transgene expression for several months in both in vitro and in vivo applications. There are EEV cloning vectors for constitutive and cumate-inducible formats available as well as pre-made EEV reporters.

Highlights

- Easy to clone format, no special plasmid production requirements
- Nonviral, non-integrating technology
- Persistent, sustained transgene expression
- Powerful CAGs promoter for constitutive, high level expression
- Works in all mammalian cell types, including primary and stem cells
- Tight, inducible Cumate Switch

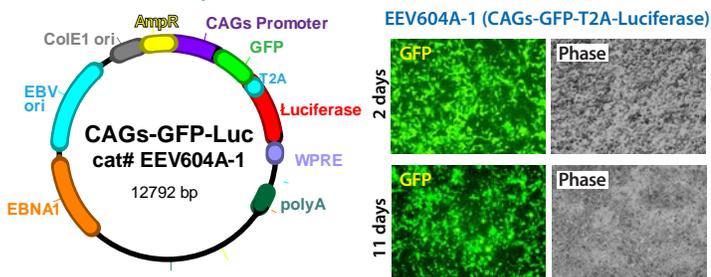
For more information:

www.systembio.com/eev

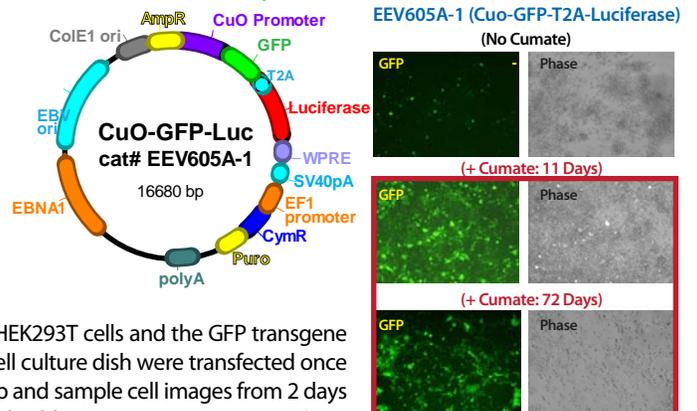
EEV Reporter Constructs

SBI has built EEV reporter plasmids featuring a GFP-T2A-Luciferase expression cassette for reporting sustained transgene expression in a constitutive format (cat# EEV604A-1) or as an all-in-one cumate inducible format (cat# EEV605A-1). Sample EEV expression for the constitutive and cumate-inducible reporter constructs are shown below.

Constitutive EEV Reporter



Cumate-inducible EEV Reporter

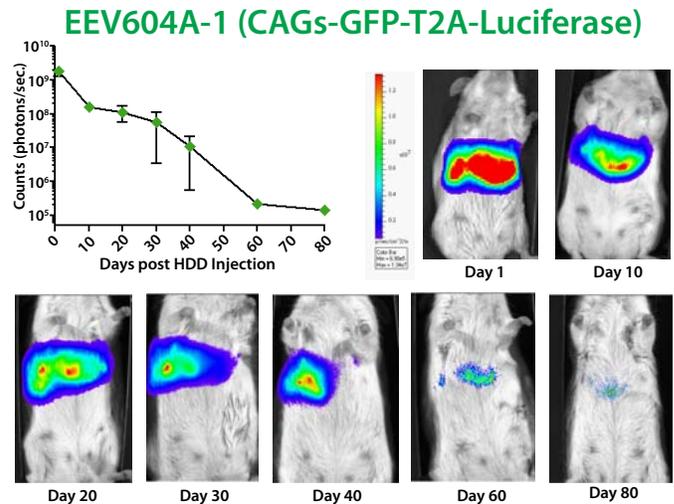


The constitutive EEV reporter construct cat# EEV604A-1 was transfected into HEK293T cells and the GFP transgene expression monitored over a period of several weeks. HEK293T cells in a 24-well culture dish were transfected once with 0.5 ug of EEV604A-1 reporter plasmid DNA. The EEV604A-1 construct map and sample cell images from 2 days and 11 days after transfections are shown in the left panels. The cumate inducible EEV reporter construct (cat# EEV605A-1) features a cumate-inducible promoter driving the expression of GFP-T2A-Luciferase as well as a constitutive EF1 promoter expressing the CymR repressor and a Puromycin selection cassette. The EEV605A-1 cumate switch inducible reporter is silent until the addition of the inducer cumate solution (Cat# QM150A-1, 10,000x concentration). Cumate was added at 1x concentration from the 10,000x stock daily at a final concentration in the medium of 300 ug/ml and cells imaged for GFP induction. The GFP induction appeared after 2 days and then expression was detected for 72 days (2.5 months) after the original transfection. The EEV605A-1 construct map and sample data for the minus cumate and the 11 and 72 day expression data are shown in the right panels.

Enhanced Episomal Vectors (EEV)

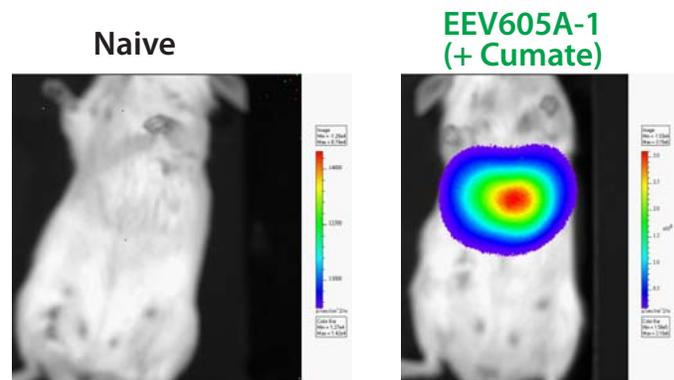
EEV Performance *In Vivo*

The constitutive EEV604A-1 CAGs-GFP-T2A-Luciferase construct (8 ug plasmid DNA) was introduced into test mice through hydrodynamic tail vein injection (HDD). This procedure leads to high plasmid DNA transfection of the livers of mice *in vivo*. The test mice (n=3) were imaged for body luminescence in the liver area post HDD from Day 1 up until Day 80. The results show that robust EEV-expressed luciferase expression is readily detectable at very high levels through day 40. Luciferase quantitative data for the test mice in graphical form as well as bioluminescent animal imaging data are shown to the right.



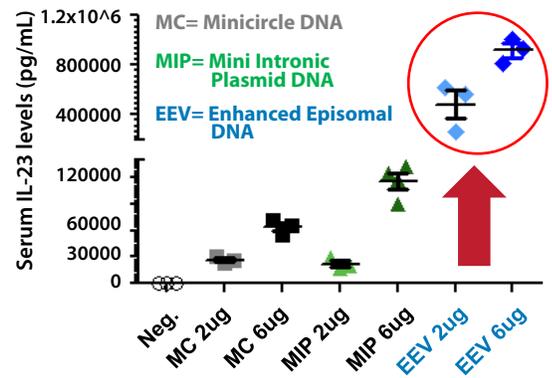
Cumate Inducible EEV Performance *In Vivo*

The Cumate inducible EEV605A-1 GFP-T2A-Luciferase reporter was also tested in mice. The EEV605A-1 CuO-GFP-T2A-Luciferase plasmid was introduced into test mice (n=3) by using 5 ug plasmid and the HDD injection procedure on Day 0. The water-soluble version of the inducer cumate compound (cat# QM150A-1) was injected by IP (1.5 mg) per animal to induce EEV expression. The mice were then imaged for luminescence activity through full body scans and liver expression levels quantitated from Day 2 through Day 10. Cumate induction of luciferase mouse images for Day 2 after cumate addition are shown to the right.



How does EEV compare to other Episomal Vectors?

There are some popular episomal expression technologies available for sustained expression *in vivo*, such as the Minicircle DNA system (Mark A. Kay, et al., Nat Biotechnol. 2010 and Mini-Intronic Platform (Lu J, et al., Mol Ther. 2013). These two different episomal technology technologies were compared against the EEV system using a mouse IL-23 cDNA cloned into the three different vectors followed by hydrodynamic tail vein injection (HDD) with subsequent monitoring of secreted mIL-23 protein levels in the serum of test mice. Two different amounts of either minicircle, mini-intronic platform or EEV plasmid DNA expressing the mIL-23 gene were utilized for these cross-comparison studies. Serum levels of mIL-23 expression were quantitated using a murine IL-23 ELISA assay 7 days after HDD injection of the various plasmid DNAs. The EEV



technology outperformed the other episomal platforms by at least 10-fold over the course of these studies up to 21 days post-HDD injection and demonstrates the ability of the EEV platform to provide sustained, long-lasting transgene expression which is critical for endpoint studies requiring weeks or even months of transgene expression.

We Also Offer Custom EEV Construct Cloning and DNA Productions Services

System Biosciences offers a wide-range of custom services to support your research, allowing you to spend less time making tools, and more time making discoveries. To learn more, visit our website at www.systembio.com/service or call us at 888-266-5066.



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