

Frequently Asked Questions: gWiz™ Expression Vectors

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1. What are the gWiz™ Expression Vectors? gWiz™ high expression vectors are a series of plasmids that have been engineered to produce high levels of transient gene expression in a wide variety of mammalian cells and tissues.

2. What are the advantages in using the gWiz™ high expression vectors?

The gWiz™ vectors contain a proprietary modified promoter followed by Intron A from the human cytomegalovirus (CMV) immediate-early (IE) gene. Although the CMV immediate early gene (IE) promoter/enhancer is the most widely used constitutive promoter for expressing high levels of transgene product in many mammalian cells and tissues, not all CMV IE gene promoter/enhancer-based expression vectors are created equal. Depending on the actual CMV IE gene sequences used and the context of the plasmid backbone upon which the expression cassette is constructed, the expression levels can vary as much as two orders of magnitude. The CMV IE promoter sequences contained in the gWIZ™ vectors have been systemically analyzed and modified. The modifications include removing the sequences that are redundant and deleterious to the high levels of expression while retaining those sequences that are of high transcriptional potency. After coupling the modified promoter with a high-efficiency synthetic transcriptional terminator, the whole expression cassette is finally constructed on a plasmid backbone that has also been streamlined and modified to accommodate the high levels of expression in mammalian cells as well as high yield of plasmid production in *E. coli*. The resulting plasmid, gWIZ™ expression vector, is capable of fully unleashing the potential of the CMV promoter and giving the highest levels of expression possible both *in vitro* and *in vivo*.

3. What reporter genes are available for the gWiz vectors?

The gWiz vectors are available with a choice of convenient reporter genes including: β -galactosidase, chloramphenicol acetyl transferase (CAT), luciferase, green fluorescent protein (GFP), secreted alkaline phosphatase (SEAP), or a blank vector containing an extensive multiple cloning site.

4. How soon after transfection can I expect to see gene expression?

This depends largely on the gene-of-interest and the cell type being transfected. However, as an example, using the gWIZ-GFP vector, GFP expression can be seen as early as 12 hrs after the onset of cell transfection.

5. How long will the expression of the transfected gene persist?

The duration of transient expression depends largely on the gene of interest and the cell type being transfected. In-house studies at Gene Therapy Systems have found gene expression of SEAP (Secreted Alkaline Phosphatase) in the blood of mice for several weeks after intramuscular injection of the encoding vector.

6. How can I replace the CMV promoter with my own cell-specific promoter?

You can easily excise the CMV promoter, enhancer and the intron A of the gWiz expression vector by using *Pst*I and *Msc*I restriction enzymes. After that, your promoter can be subcloned into the vector using standard cloning techniques.

7. Do the gWiz plasmids have the TPA response element?

The gWiz high expression vectors do not contain the TPA response element.

8. Can I use kanamycin for antibiotic selection in achieving stable transfection in mammalian cells?

The kanamycin resistant gene encoded in the gWiz vectors is only to use for bacterial selection. It is not active in mammalian cells.

9. Can I use the gWiz plasmids for stable transfection?

gWIZ plasmids are optimized for high-level transient expression studies. However, if you want to carry out stable transfection with gWIZ plasmids, the fastest way that it can be achieved is to do a co-transfection using gWIZ vector mixed with a second plasmid expressing a selection gene (e.g. neomycin resistance). In our studies, the optimal ratio of gWIZ to selection plasmid was 5:1. Twenty percent of neomycin resistant cell lines obtained this way also had the gene of interest stably integrated. In addition, a series of stable expression vectors were constructed using the gWiz backbone.

10. What type of polyadenylation sequence is included in the gWIZ vectors? The gWIZ vectors contain a modified rabbit β -globin polyadenylation sequence.

11. What concentration of kanamycin do I need in my medium to select stable transfectants using the gWIZ vector's kanamycin selection gene?

We recommend using a kanamycin concentration of 100 μ g/ml.