ECO→TECH
Biotechnology

EndoGenius Inducer Assay

A novel tool for endogene overexpression

version 23.1

1. A brief history of overexpression studies

The roles of specific genes in biological, physiological and pathological processes are examined by up- or downregulation of their expressions in various research fields including molecular biology, genetics, stem cell, gene therapy, and biotechnology.

The theoretical basis for using overexpression as a genetic tool predates molecular cloning.

The early findings demonstrating that gene expression level is important for normal gene function stem from karyotype analyzes showing that aneuploidies are responsible for human genetic syndromes (1) and mutant phenotypes in Drosophila and plants (2, 3).

Other findings pointing to the importance of gene expression level were obtained as a result of classical studies on bacteriophage morphogenesis. Although these phage studies did not include overexpression, they demonstrated the understanding that expression level is important for bacteriophage morphogenesis and may therefore be important for other biological processes as well.

These insights formed the conceptual basis for the development of overexpression tools, with the realities that balanced gene expression is important and that changes in copy number can result in mutant phenotypes. As a natural extension of this concept, it has been recognized that targeted overexpression of specific genes can be a useful tool for associating genes to biological pathways (3).

With the development of recombinant DNA technology in the 1970s, genes were cloned and transferred to plasmids or other vectors, and very comprehensive vector libraries were created (4). These vectors were transferred to cells with the help of chemical or viral agents, and the desired gene was expressed at a high level in cells (Figure 1) (5).

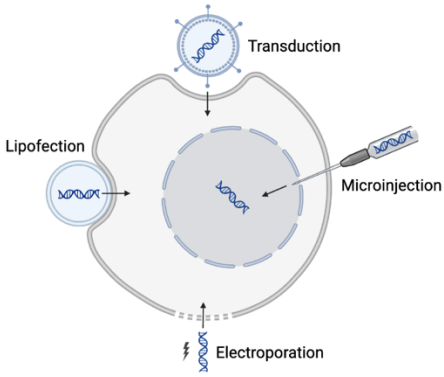

Figure 1. Transfer of vectors into the cells via distinct tools. The figure is created in BioRender.com

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Important implications for human health have been implemented from the lessons learned from overexpression studies, and these implications have changed our understanding of the causes and treatment of diseases.

First, there are numerous examples of human diseases directly caused by increased gene expression, sometimes accompanied by gene amplification (6, 7).

Moreover, even if overexpression does not directly cause diseases, changes in gene expression patterns or levels can contribute to phenotypic variation, diversity, and evolution (8). For example, copy number variations (CNVs) can cause familial diseases in humans and possibly contribute to more complex disease phenotypes (9).

Third, the successful application of systematic overexpression studies in organisms such as yeasts, Drosophila, and Arabidopsis strongly suggested that similar collections of systematic overexpression of human genes will be valuable fundamental research tools in cell culture systems to uncover new therapeutic applications.

Important milestone studies such as generation of induced pluripotent stem cells (10) and generation of neurons from fibroblasts (11) are important examples of the application of combinatorial targeted overexpression to potential therapeutic use. Finally, the understanding that overexpression can cause a variety of phenotypes, including diseases in humans, highlights the importance of establishing correct expression levels in gene therapy strategies.

Considering above mentioned reasons, it is an important requirement to develop methods and tools for overexpression of genes.

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2. Currently available overexpression tools have certain drawbacks

Ectopic overexpression via plasmids has significant shortcomings;

- When genes are overexpressed with plasmids, gene expression levels may reach supra-physiological doses and supra-pathological doses, and the pathological conditions cannot be evaluated on a realistic scale (3).

- Overexpression of very large genes such as DMD, TTN and etc. is very difficult using current tools due to the fact that vectors often have very limited insert sizes.

- Alternative splice is an important phenomenon in nature, and up to 95% of the multi-exon human genes are subject to alternative splicing (12). Therapeutic gene entry is often limited to the intracellular delivery of a splice variant of a gene. However, for the proper regulation of cellular processes, it can be extremely important to overexpress all splice variants of a gene of interest in the target cell at the correct rate. The importance of correct stoichiometric expression of all splice variants of a gene has been demonstrated for angiogenesis in a mouse model. It has been shown that induction of endogenous gene expression of VEGF-A results in the formation of more mature vessels compared to exogenous introduction of the gene encoding only one splice variant of VEGF-A (13). It is also possible to target a common promoter sequence for different genes of a gene family. Therefore, the expression of multiple genes can also be easily altered using a single tool.

- *In vivo* delivery of overexpression plasmids to the target tissue stays as an important obstacle when therapeutic strategies are developed due to their larger sizes and lower transfer efficiency to the target cells.

*For these reasons, **regulation of endogenous gene expression** is a new and exciting approach in medical research.*

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3. How EndoGenius Inducer Assay differs from currently available tools

EndoGenius Inducer Assay utilizes oligonucleotides to targeted guidance of certain epigenetic regulators, which are already present within the cell, to the specific gene promoters to overexpress specific endogenes.

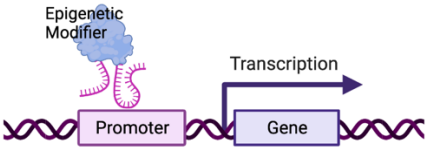

Figure 2. General mechanism of action of EndoGenius Inducer Assay. The figure is created in BioRender.com

EndoGenius Inducer Assay includes Control Mix, which is equivalent to control plasmids, and the necessary carrier agent (Encapsulation Buffer), which is an optimized transfection reagent for effective delivery of Active Mix into the cells. Therefore, EndoGenius Inducer Assay eliminates the need for purchasing additional controls and transfection reagents. EndoGenius Inducer Assay is All-in-One solution.

Endogenous gene overexpression via EndoGenius Inducer Assay has significant **advantages**;

√ Delivery of only short oligonucleotide sequences compared to plasmids provide more effective transient overexpression of target genes. Fluorescent labeled Active Mix of EndoGenius Inducer Assay can be delivered to cells with almost 100% efficiency (Figure 3).

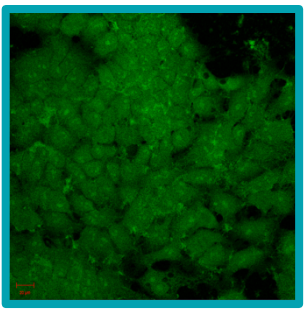

Figure 3. EndoGenius Inducer Assay helps overexpression of target gene with almost 100% efficiency.

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√ Induction of endogenous gene overexpression using specific EndoGenius Inducer Assay results in expression of all splice variants that is expressed in that specific cell or tissue.

For example, Gene A has 2 splice variants and both of them are expressed in SCC-9 cells. Overexpression of Gene A in cells *in vitro* using a plasmid carrying isoform 1 of Gene A only results in overexpression of isoform 1 (Figure 4A), but not in overexpression of isoform 2 (Figure 4B).

However, EndoGenius Inducer Assay can induce overexpression of both splice variants (Figure 4A-B), which provides more relevant and robust results when analyzing the effects of a specific gene on cellular features.

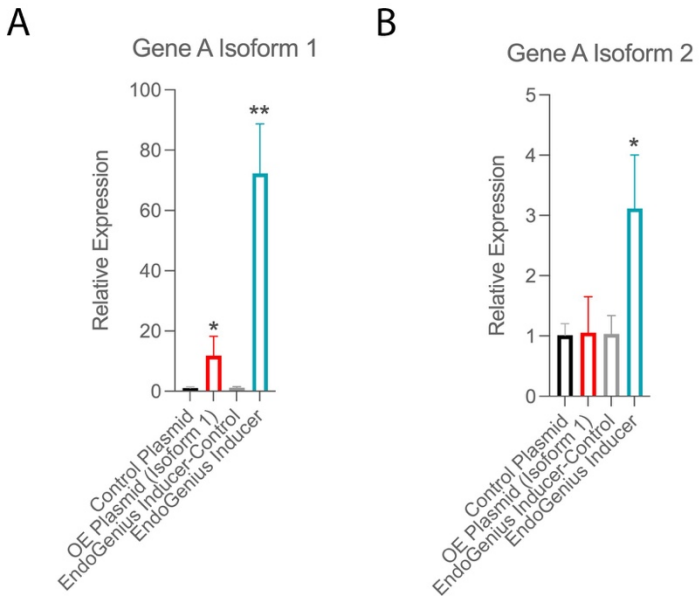

Figure 4. A. Relative expression of isoform 1 of Gene A when cells are transfected with an overexpression plasmid carrying isoform A clone or cells were treated with EndoGenius Inducer Assay. B. Relative expression of isoform 2 of Gene A when cells are transfected with an overexpression plasmid carrying isoform A clone or cells were treated with EndoGenius Inducer Assay.

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√ EndoGenius Inducer Assay allows both overexpression of even of the largest genes in the genome (Figure 5) and overexpression of all cell-specific expressed variants.

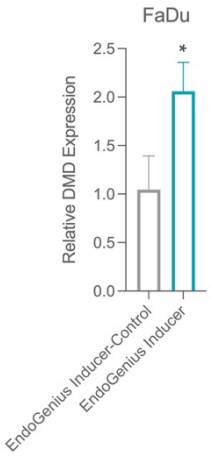

Figure 5. Relative expression of DMD, one of the largest genes in the human genome, when induced with specific EndoGenius Inducer Assay in vitro.

√ Utilization of EndoGenius Inducer Assay allows specific gene overexpression (Figure 6A) with minimal off-target effects (Figure 6B). It is quite easy to carry out an overexpression assay to see functional effects of overexpressing an endogenous gene. For example, overexpression of a specific oncogene results in increase in viability (Figure 6C).

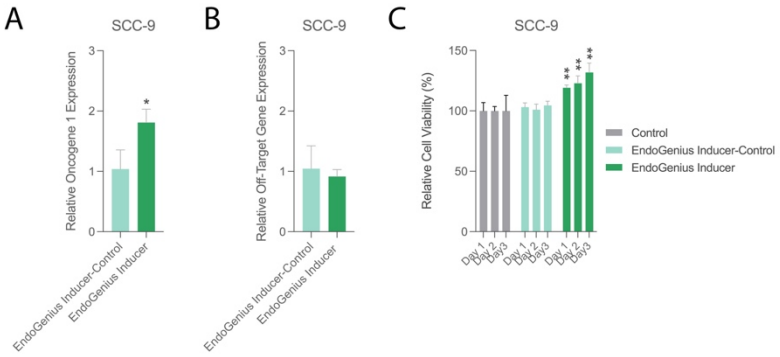

Figure 6. A. EndoGenius Inducer specifically induce significantly overexpression of Oncogene 1, B. with no alteration in other genes. C. Overexpression of Oncogene 1 results in significant increase in cell viability.

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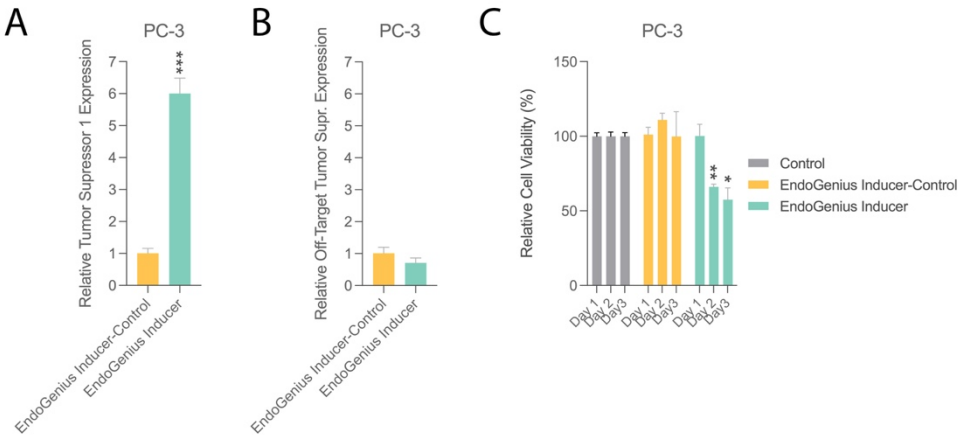
On the other hand, overexpression of Tumor Suppressor Gene 1 using EndoGenius Inducer Assay (Figure 7A), with no significant change in the expression of another tumor suppressor gene from the same gene family (Figure 3B), results in reduced cell viability (Figure 3C).

Figure 7. A. EndoGenius Inducer specifically induce overexpression of Tumor Suppressor Gene 1, B. with no alteration in another tumor suppressor gene from the same gene family. C. Overexpression of Tumor Suppressor 1 results in significant decrease in cell viability.



Conclusion

- √ EndoGenius Inducer Assay helps overexpression of endogenes at transcription level and is an all-in-one assay, which necessitates no control plasmids, vectors, etc., transfection reagents.
- √ It provides delivery efficiency similar to viral transduction with no need for additional infrastructure for virus handling.
- √ Besides, it alters the expression of all splice variants that are expressed in that specific cell or tissue.
- √ Most importantly it eliminates the need for cloning for overexpression studies, which enables overexpression of even of the largest genes in the genome.

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