

B-MO'S
**TIP OF THE
MONTH**

OCT 2016

BMOGEN'S THE VALUE OF INSULATION

Making Expression Vectors that Really Work





B-MoGen's The Value of Insulation Making Expression Vectors that Really Work

There are numerous chemical, physical and viral-based methods for gene transfer into cells in culture. These methods have helped revolutionize biological research and have provided the biotechnology field with the tools needed to make useful cell lines, producing proteins, vaccines and much more.

However, not enough attention has been paid to ensuring that transgenes maintain high-level expression over a long time. In many cases, loss of transgene expression is caused by so-called position effect variegation in which heterochromatin migrates into and over transgenes in some cells in a population, thus silencing them.¹ This seems to be especially prevalent in primary cells. The position effect variegation is a clear problem for many basic research studies and protein production projects using transfected or transduced cells where long-term expression is desired.

At B-MoGen we have invested in development of transposon vectors that are easy to modify, easy to introduce efficiently into cells, have large carrying capacity, and can uniformly maintain expression of many transgenes over a long period of time in culture.²

We have found that inclusion of tandem insulator sequences from the chicken hypersensitive site-4 (cHS4) flanking our transgenes remarkably preserved long-term expression of transposon vectors expressing four or more transgenes. **SEE FIGURE 1.** Indeed, we observed that vectors with several transgenes within one construct are more likely to be silenced over time. Thus, our insulated transposon transgenes can result in a tremendous improvement in long-term expression of multigene vectors.

The potential applications for multigene transfer vectors in basic and applied research are many. This includes studies on cancer development, cellular reprogramming (e.g. induced pluripotent stem cell generation), protein production, and more.

FIGURE 1A.

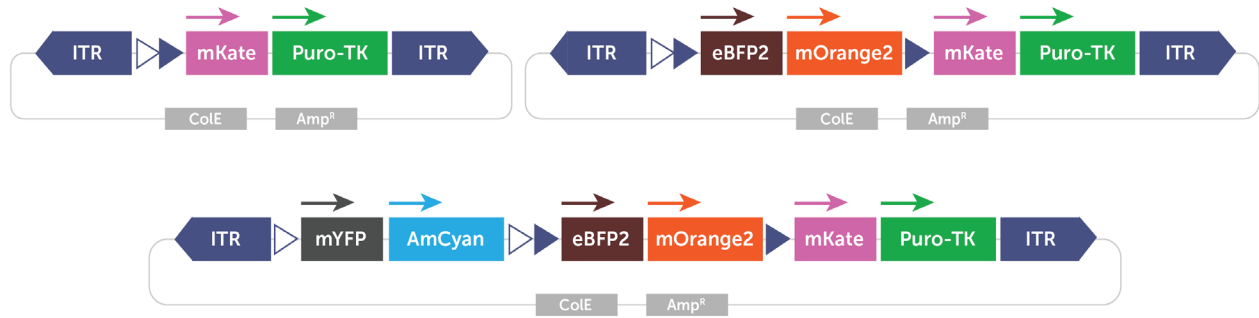


FIGURE 1B.

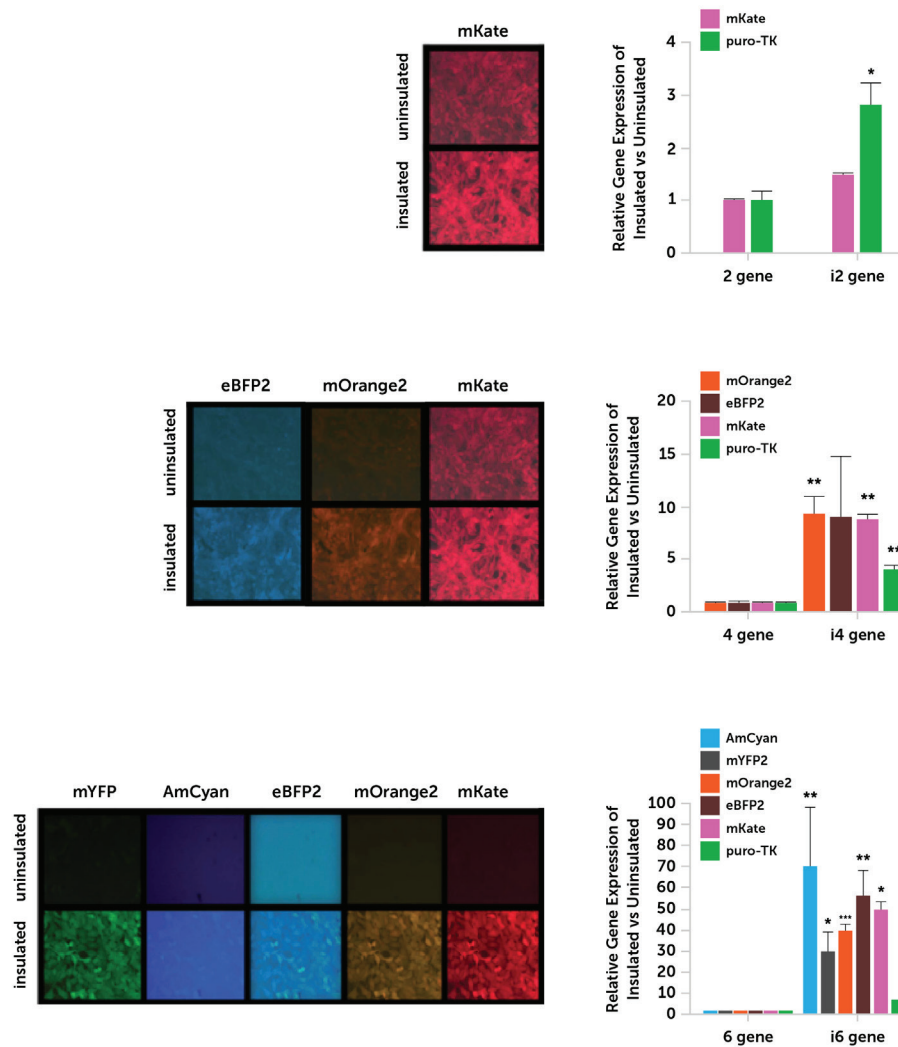


FIGURE 1. EXPRESSION OF STABLY INTEGRATED MULTIGENE TRANSPOSON VECTORS IN NIH 3T3 CELLS.

- Diagram of vectors expressing one, three, or five fluorescent proteins along with puro-tk fusion gene.
- Representative fluorescence photomicrographs and QRT-PCR analysis of stably integrated insulated and uninsulated multigene vectors. Expression levels are normalized to uninsulated vector gene expression for direct comparison of insulated and uninsulated vectors. P-values were calculated using a two-tailed unpaired t-test ($P > 0.0001$ ***, $P > 0.001$ **, $P > 0.01$ *) . . .

REFERENCES

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