

miTarget™ miRNA Target Sequence 3' UTR Expression Clones

Uncomplicated

GeneCopoeia functional analysis tools facilitate miRNA studies with genome-wide miTarget™ miRNA target sequence 3' UTR expression clones. miTarget clones are available in two mammalian expression vector systems.

- ◆ All 3' UTR sequences were obtained from public domain databases.
- ◆ 3' UTR sequences are inserted downstream of the coding sequences.
- ◆ The firefly luciferase (non-secreted) and *Gaussia* luciferase (secreted) reporter genes are controlled by an SV40 promoter.
- ◆ Luciferase expression is regulated by binding of the targeting miRNA to the 3' UTR target sequence; luciferase activity is quantified with a colorimetric assay.
- ◆ Vectors include *Renilla* luciferase or alkaline phosphatase which can be used as a normalization reporter gene.

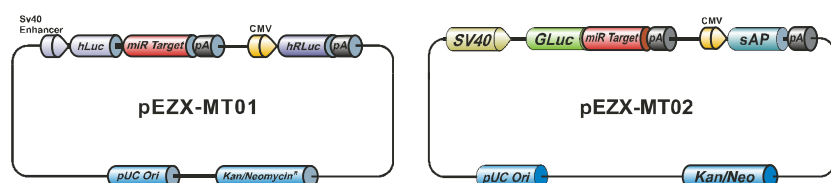


Figure 4. Vector features for human miRNA target sequence expression clones.

The vectors offer different combinations of reporter and tracking genes to match different assay requirements.

Vector	Reporter gene	Tracking gene	Advantage
pEZX-MT01	Firefly luciferase	<i>Renilla</i> luciferase	Assays can be performed on cell lysates.
pEZX-MT02	<i>Gaussia</i> luciferase	Alkaline phosphatase	Target cells are not destroyed to perform enzymatic assays.

Discovery simplified

- The regulatory effect of a particular miRNA on its potential target is assessed with an enzymatic assay for firefly or *Gaussia* luciferase.
- The miRNA 3' UTR expression constructs transcribe chimeric mRNAs consisting of a luciferase coding sequence and a 3' UTR target sequence.
- The reporter enzymatic assays are conducted with either firefly luciferase (non-secreted) or *Gaussia* luciferase (secreted).