miRNAsguide the RISC to target messages that are subsequently cleaved or translationally silenced.

![miRNA Biogenesis and function](image)

**Figure 1. miRNA Biogenesis and function**
Synthetic miRNA molecules based on predicted mature miRNA sequence are sometimes used. Despite their optimized design criteria, synthetic miRNAs underscore the importance of primary miRNA in its native expressed form. The primary miRNA contains critical biological components involved in mature miRNA expression and cellular processing, and is often processed into several mature miRNA molecules.

Cell Biolabs’ microRNA precursor vectors express each individual human miRNA precursor in its native context while preserving putative hairpin structures to ensure biologically relevant interactions with endogenous processing machinery and regulatory partners, and that leads to properly cleaved microRNAs. Each individual miRNA precursor is cloned between BamHI and Nhe I sites (Figure 2).

The pEP-mir vectors contain the following features:
- **miRNA precursor** – 100 bp stem loop precursor in its native context flanked by a human intron sequence to preserve the putative hairpin structure and proper endogenous processing
- **EF-1α Promoter** - ensures a high level of expression in mammalian cells
- **Puromycin resistance marker** - to monitor cells positive for expression and stable selection
- **SV40 polyadenylation signal** - enables efficient termination of transcription
- **pUC origin** - for high copy replication and maintenance of the plasmid in *E. coli*
- **Ampicillin resistance gene** - for selection in *E. coli*

![Figure 2. Schematic representation of pEP-miR expression vector.](image-url)

miRNA precursor sequence:

```
1 ttcgaggatcc tgggtgggtc tggggtcggg ggtgctgaga aggggagtga gggcttctcg
61 ggtgccccag cttctcgctt ccctatgaga ttcctgccgc tggacccctc cactctgctg
121 tggcctatgg cttttcattc ctatgtgatt gctgtcccaa actcatgtag ggctaaaagc
181 catgggctac agtgaggggc gagctccttc tcctgcgcag ctgcacctcc catgggacca
241 ggttcggagc cagccaccaa ggggcaccag aaggaggctt tgcttggggg tggggcatca
301 cgggatcgcgt agctcga
```
Methods

1) Bacterial culture: the microRNA precursor construct is provided as bacterial glycerol stock. Individual colonies can be obtained by culturing in an LB-ampicillin plate.
2) Plasmid isolation: we recommend EndoFree Plasmid Kits (QIAGEN).
3) Transfection into target cells: we recommend Lipofectamine 2000 (Invitrogen).
4) Stable selection: 48 hrs post-transfection, select stable clones in 1-10 µg/mL Puromycin-containing medium.

References

1. microRNA sequences listed in Sanger’s miRBase (http://microrna.sanger.ac.uk/sequences/).

Recent Product Citation


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