

## **Transposon vectors**

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NBRP provides the Minos and Sleeping Beauty transposon systems for germline transgenesis (Sasakura et al., 2003, PNAS 100, 7726-7730 and Hozumi et al., 2013, Dev Dyn 242, 30-43). Following is an explanation of Minos.

The Minos system is usually used to insert a reporter gene expression cassette into the Ciona genome to create transgenic lines. pMiLRneo is the basic cloning vector for Minos. This vector has a neomycin-resistant gene between the inverted repeats (Figure 1), and the NeoR should be removed and replaced by a reporter cassette. Following is the method by which In-fusion can be used to create Minos vectors:

Amplify your promoter>reporter gene unit in the pSP vector with the primers:

```
CAGAGCTGGGGGATCCGATTAGGTGACACTATAG
TAGAACTAGTGGATCCGACTCACTATAGGGAGAC
```

Digest pMiLRneo with BamHI.

Electrophorese the above DNA to isolate the appropriate bands.

Perform an In-fusion reaction.

If your reporter cassette does not express a reporter gene after metamorphosis, or if your reporter cassette does not have a reporter gene visible in live animals, adding a visual marker in your Minos vector will facilitate the screening of transgenic lines (you can screen transgene-positive animals from immotile juveniles).

pMiFr3dTPOR can be used for this purpose (Figure 2). When you use the vector to insert your reporter cassette, please use the following method:

Amplify the cassette with the following primers:

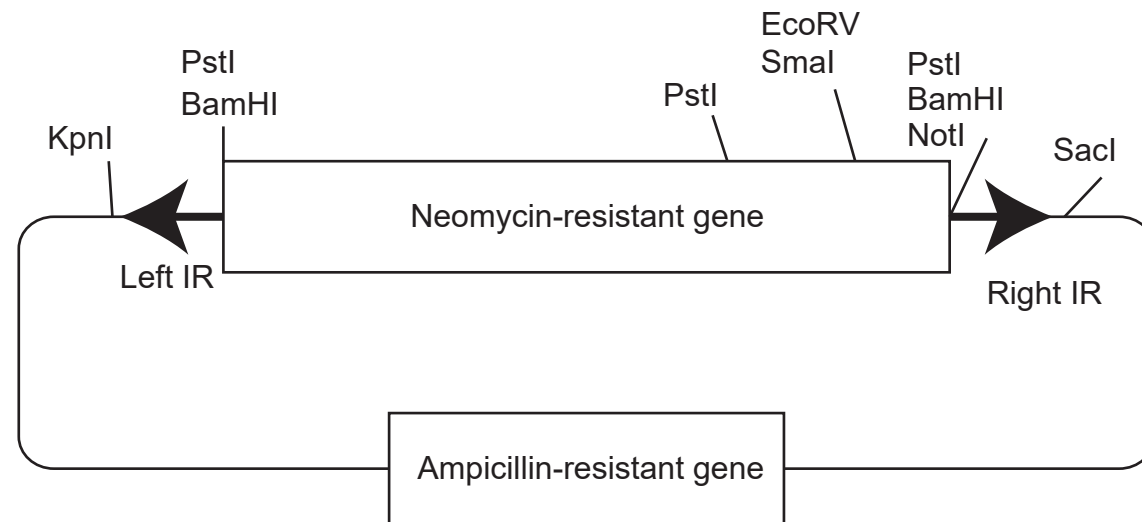
```
GATCCGAAAATGGATCGATTAGGTGACACTATAG
TTCGGGAGCTTGGATGACTCACTATAGGGAGACC
```

Linearize pMiFr3dTPOR with EcoRV.

After purification, insert the cassette by In-fusion. The direction of your reporter and that of DsRed will be the same. If you wish to reverse the orientation, modify the above primers.

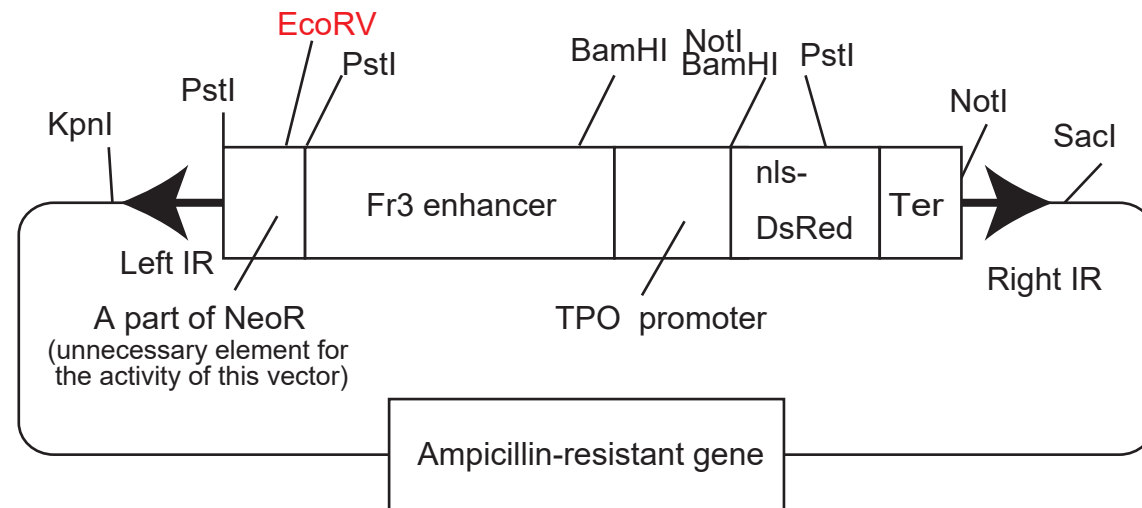
NBRP provides several Minos markers, and you can use a vector appropriate for your experiments. For example, pMiFr3dTPOR has bright RFP expression in the endostyle (Figure 2), which would mask your reporter expression there. In this case, you should select a different Minos vector to add a visual marker appropriate for your experiment.

Figure 1 Structure of pMiLRneo



Caution: KpnI and SacI must not be used.

Figure 2 Structure of pMiFr3dTPOR



Expression pattern of DsRed (magenta) from Fr3dTPOR cassette.

