

Transposon vectors

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NBRP provides Minos and Sleeping beauty transposon systems for germ-line transgenesis (Sasakura et al., 2003, PNAS 100, 7726-7730 and Hozumi et al., 2013, Dev Dyn 242, 30-43). The following is the explanation about Minos.

Minos system is usually used for inserting a reporter gene expression cassette into Ciona genome for creating transgenic lines. pMiLRneo is the basic cloning vector for Minos. The vector has neomycin resistant gene between the inverted repeats (Figure 1), and the NeoR should be removed and instead reporter cassette should be inserted. The following is the method to use In-fusion for creating Minos vectors:

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

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CAGAGCTGGGGGATCCGATTTAGGTGACACTATAG
TAGAACTAGTGGATCCGACTCACTATAGGGAGAC
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Digest pMiLRneo with BamHI.

Electrophorese the above DNA to isolate the appropriate bands.

In-fusion reaction.

If your reporter cassette does not have the activity to express 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 screening of transgenic lines (you can screen transgene positive animals from immotile juveniles). pMiFr3dTPOR can be used for the purpose (Figure 2). When you use the vector for inserting your reporter cassette, please use the following methods:

Amplify the cassette with the following primers:

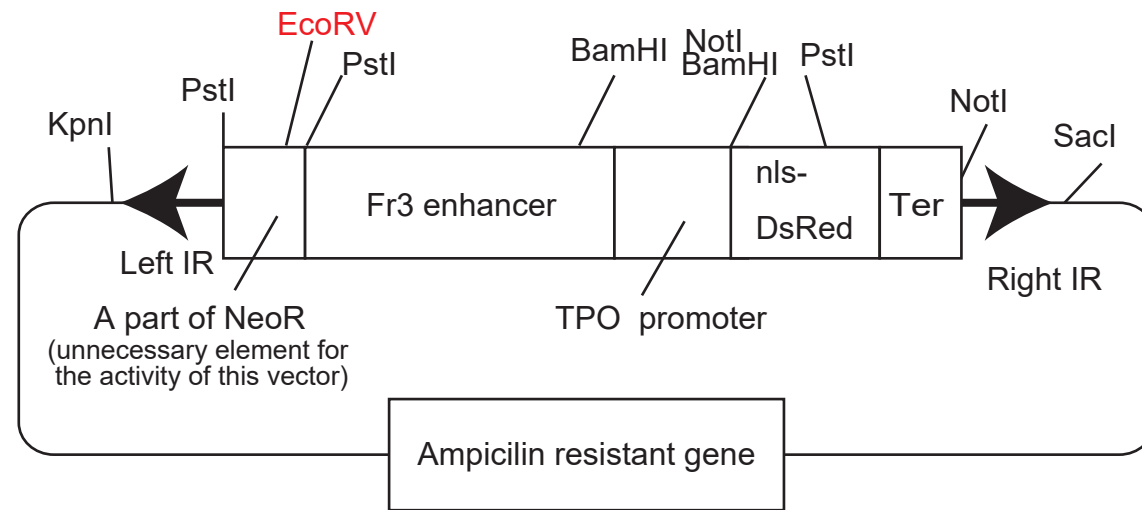
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GATCCGAAAATGGATCGATTTAGGTGACACTATAG
TTCGGGAGCTTGGATGACTCACTATAGGGAGACC
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Linearize pMiFr3dTPOR with EcoRV.

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

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

Figure 2 The structure of pMiFr3dTPOR



Expression pattern of DsRed (Magenta) from Fr3dTPOR cassette.

