COSMO BIO CO., LTD.
Inspiration for Life Science

Catalog No. CSR-CT-NU-002-1

Tol1-based transgenesis vector

Product Description	Donor and helper plasmids for transgenesis in vertebrates. The donor plasmid contains terminal regions of the Tol1 element and multicloning sites for integration of a gene to be transferred to the host chromosome. The helper plasmid carries the transposase gene of the Tol1 element. Tol1 is a DNA transposon identified in the medaka fish and demonstrated to be active in various vertebrate species.
Volume	1 μg
Formulation	Filter paper contains dried plasmid DNA at positions marked with circles.
Storage & Stability	Store at room temperature. Stable for 1 year from the date of shipment.
Reference	1) Koga A, Cheah FS, Hamaguchi S, Yeo GH, Chong SS (2008). Germline transgenesis of zebrafish using the medaka Tol1 transposon system. Dev Dyn. 237: 2466-2474. 2) Koga A, Higashide I, Hori H, Wakamatsu Y, Kyono-Hamaguchi Y, Hamaguchi S (2007). The Tol1 element of medaka fish is transposed with only terminal regions and can deliver large DNA fragments into the chromosomes. J. Hum. Genet. 52: 1026-1030.

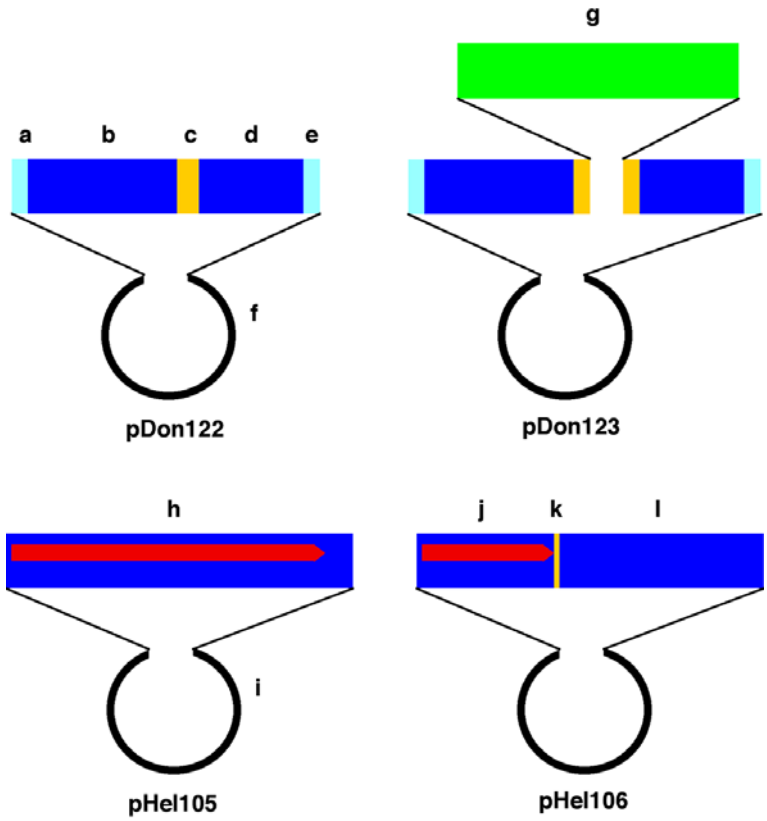
Components

pDon122: A vacant donor plasmid.

pDon123: Donor plasmid carrying the GFP gene.

pHel105: Helper plasmid. Its vector portion is pCS2+, having the CMV promoter for *in vivo* expression of the transposase gene and the SP6 promoter for *in vitro* synthesis of the transposase mRNA.

pHel106: A defective helper which is useful for negative control experiments especially when you want to know the net transformation efficiency.



a.. Target site duplication
CCTTTAGC

b. *Tol1* left arm
nt. 1-157 of GenBank D42062

c. Multicloning sites
GATCC GAATTC GATATC GGTACC CTGCAG TCTAG
***Bam*HI *Eco*RI *Eco*RV *Kpn*I *Pst*I *Xba*I**

d. *Tol1* right arm
nt. 1750-1855 of GenBank D42062

e. Tartget site duplication
CCTTTAGC

f. pUC19
GenBank U55763.
nt. 391-460 were changed to:
CCAGTGAAT GTCGAC CATGC AAGCTT GGCGTAAT
***Hinc*II *Hind*III**

g. CMV promoter + EGFP + Poly(A) signal
nt. 1-1632 of GenBank U55763
Inserted at the EcoRV site of the muticloning sites.

h. Entire coding sequence for transposase
nt. 31-2817 of GenBank AB264112

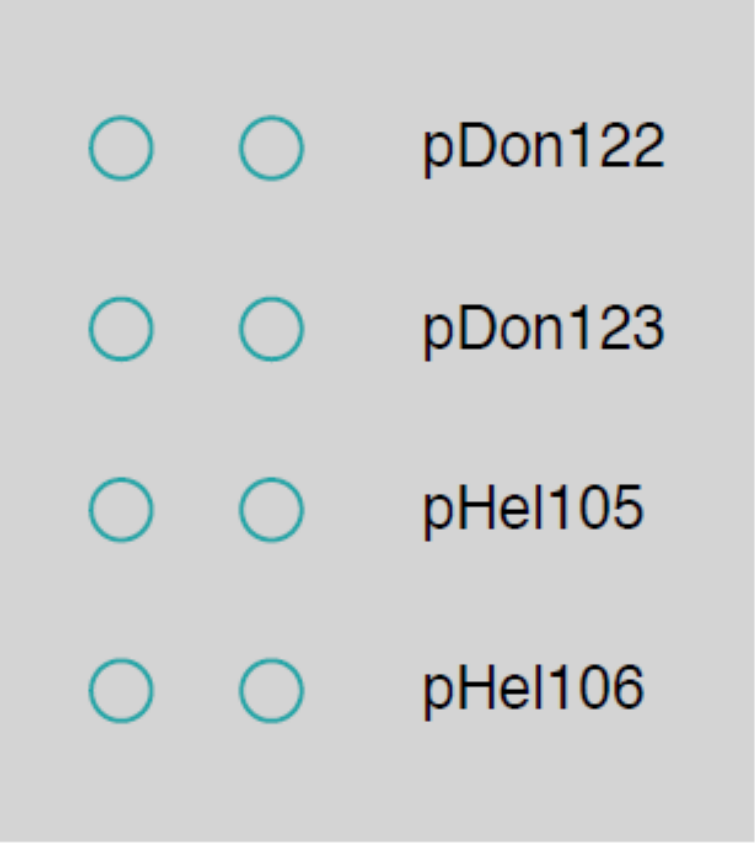
i. pCS2 (+), carrying CMV promoter, SP6 promoter, MCS 1, 3' UTR, and MCS2.

j. nt. 31-995 of GenBank AB264112

k. nt. 996-1001 (ATGAAA for methionine and lysine) were changed to two stop codons (TAGTAA).

l. nt. 1002-2817 of GenBank AB264112

How to recover plasmid DNA



1. Cut out one of the circles of the paper and immerse it in water or TE in a microfuge tube. Other circles are for backup.
2. Mix by tapping.
3. Centrifuge for 1 minute at >10 krpm.
4. Transform competent bacterial cells (commonly used strains, such as JM109, DH5α and XL1-Blue) with a small amount of supernatant.
5. Spread the bacteria on an LB/agar plate containingl ampicillin, and incubate the plate at 37°C C for >12 hours.
6. Pick up a single colony.
7. Amplify bacteria in liquid media.
8. Extract plsmid DNA by the standard method.

For research use only. Not for clinical diagnosis.

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