

pJTI™ R4 EXP CMV-TO EmGFP pA Vector

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Rev. 2.0

Component	Amount	Storage
pJTI™ R4 EXP CMV-TO EmGFP pA vector	100 µg in TE buffer, pH 8.0*	-20°C

* TE Buffer, pH 8.0: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

Description

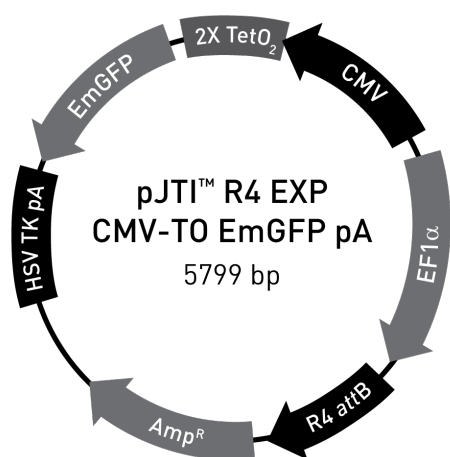
The pJTI™ R4 EXP CMV-TO EmGFP pA vector is a positive control vector for assessing the success of a retargeting reaction in a Jump-In™ T-REx™ parental cell line. When co-transfected with the integrase vector (pJTI™ R4 Int vector, included in the Jump-In™ T-REx™ parental kits) into a Jump-In™ T-REx™ parental cell line containing the genomic R4 site, the Emerald Green Fluorescent Protein (EmGFP) is expressed under the control of the TET repressor. After induction with doxycycline, successfully integrated cells will fluoresce green.

FAST FACTS

- For detailed information on the use and propagation of pJTI™ R4 vectors, refer to the user guide for your Jump-In™ T-REx™ parental cell line available on our website. Visit www.lifetechnologies.com to search for the part number of the Jump-In™ T-REx™ parental kit and select manuals.
- We highly recommend** including the pJTI™ R4 EXP CMV-TO EmGFP pA vector in your Jump-In™ T-REx™ retargeting experiment as a positive control along with negative controls (no plasmid DNA, no integrase vector) to visualize the results and optimize the retargeting conditions.

Map of pJTI™ R4 EXP CMV-TO EmGFP pA vector

The pJTI™ R4 EXP CMV-TO EmGFP pA vector (5799 base pairs) is designed for inducible expression of EmGFP after retargeting into the genomic R4 site of a Jump-In™ T-REx™ platform cell line. The vector is intended for use as a positive control for retargeting experiments. Co-transfection with the integrase vector pJTI™ R4 Int into Jump-In™ T-REx™ parental cell lines containing the genomic R4 site results in Geneticin® resistance for selected retargeted cells. The complete sequence of the pJTI™ R4 EXP CMV-TO EmGFP pA vector is available for downloading at www.lifetechnologies.com or by contacting Technical Support.



pJTI™ R4 EXP CMV-TO EmGFP pA

5799 nucleotides

2X TetO₂: bases 156–195 (c)

CMV promoter: bases 196–782 (c)

EF1α promoter: bases 902–2080

R4 *aatB*: bases 2159–2453

Ampicillin resistance gene (Amp^R): bases 2605–3462

HSV TK polyA: bases 4663–4934 (c)

EmGFP: bases 5067–5783 (c)

*(c): complementary strand

Technical Support

For assistance, contact our technical support team at drugdiscoverytech@lifetech.com or 760-603-7200 (enter 3 for “know your party’s extension”, then enter 40266).

For Research Use Only. Not for use in diagnostic procedures.

Features of pJTI™ R4 EXP CMV-TO EmGFP pA vector

The pJTI™ R4 EXP CMV-TO EmGFP pA vector contains the following elements. All features have been functionally tested.

Feature	Benefit
2X TetO ₂	Tetracycline operator sequence (TO) consisting of two tandem 19 nucleotide repeats that serve as a binding site for Tet repressor homodimers
CMV promoter	Human cytomegalovirus immediate-early (CMV) promoter/enhancer for high-level expression in a wide range of mammalian cells
EF1α promoter	Drives the high level expression of the Geneticin® resistance gene following the retargeting into the genomic R4 site of the parental Jump-In™ T-REx™ cell line
R4 attB site	Allows R4 integrase-mediated integration into the complementary genomic R4 attP site in the parental Jump-In™ cell line
Ampicillin resistance	Allows the selection and propagation of the vector in <i>E. coli</i>
HSV TK polyA signal	The Herpes Simplex Virus thymidine kinase polyadenylation signal for proper termination and processing of the recombinant transcript
EmGFP	Emerald Green Fluorescent Protein for fluorescent detection of retargeted cells

Propagating pJTI™ R4 EXP CMV-TO EmGFP pA vector

To propagate and maintain the pJTI™ R4 EXP CMV-TO EmGFP pA vector, we recommend using 10 ng of the vector to transform a *recA*, *endA* *E. coli* strain such as TOP10F', DH5α™-T1^R, TOP10, or equivalent. Select transformants on LB plates containing 50–100 µg/mL of ampicillin. Be sure to prepare a glycerol stock of a transformant containing the plasmid for long-term storage.

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