invitrogen™
by life technologies™

pJTI™ R4 Exp CMV EmGFP pA Vector

Catalog no. A14146 **Shipping:** Dry ice **Storage:** –20°C

Protocol part no. A14146PPS (MAN0005354) **Rev. date:** 11 October 2011

Table of Contents

Contents and Storage

Component	Part no.	Amount	Storage
pJTI™ R4 Exp CMV EmGFP pA vector	A14146	200 µL at 0.5 µg/µL in TE buffer, pH 8.0	–20°C

Overview

The pJTI™ R4 Exp CMV EmGFP pA vector is a positive control vector for assessing the success of a retargeting reaction in a Jump-In™ parental cell line. When co-transfected with the integrase vector (pJTI™ R4 Int vector) included in the Jump-In™ parental kits into a Jump-In™ parental cell line containing the genomic R4 site, the EmGFP will be expressed and successfully integrated cells will fluoresce green.

Successful retargeting of Jump-In™ parental cell lines like the Jump-In™ GripTite™ HEK293 kit (Cat no. A14150) is dependent on a variety of factors such as

- Transfection efficiency
- Cell confluency
- Antibiotic selection conditions
- Quality and concentration of DNA
- Retargeting vector to integrase vector ratio

We strongly recommend including the pJTI™ R4 Exp CMV EmGFP pA vector in your Jump-In™ retargeting experiment as a positive control along with negative controls (no plasmid DNA, no integrase vector) so that you can easily visualize the results and optimize the retargeting conditions.

Purpose of this User Guide

This user guide provides a brief overview of the vector's functional elements and how to propagate the vector. This user guide **does not** provide detailed instructions for culturing the Jump-In™ parental cells, retargeting the Jump-In™ parental cells, or characterization and quality control of retargeted Jump-In™ cells. For more information, refer to the detailed manual for a Jump-In™ parental cell line kit manual on our website (**www.lifetechnologies.com**) or by contacting Technical Support.

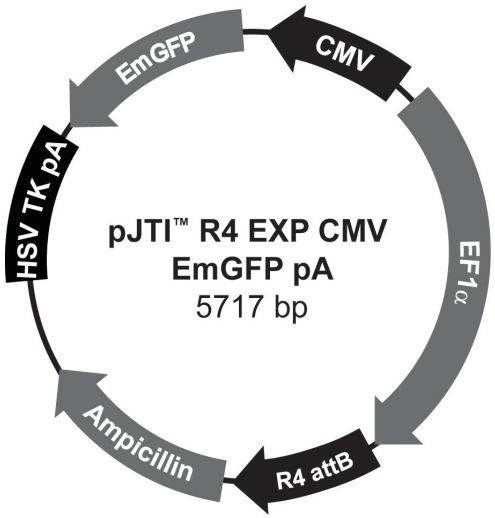
Technical Support

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).

pJTI™ R4 Exp CMV EmGFP pA Vector

Map of pJTI™ R4 Exp CMV EmGFP pA

The pJTI™ R4 Exp CMV EmGFP pA vector (5,717 base pairs) is designed for the expression of Emerald Green Fluorescent Protein (EmGFP) after retargeting into the genomic R4 site of a Jump-In™ platform cell line. The vector is intended for use as a positive control for retargeting experiments. The complete sequence of pJTI™ R4 EXP CMV EmGFP pA is available from **www.lifetechnologies.com** or by contacting Technical Support.



Features of pJTI™ R4 EXP CMV EmGFP pA
5717 nucleotides

CMV promoter: bases 142–729 (c)*
EF1α promoter: bases 849–2027
R4 *aat*B: bases 2106–2400
Ampicillin resistance gene: bases 2552–3409
HSV TK pA: bases
EmGFP: bases 4985–5701 (c)

*(c): complementary strand

Life Technologies Corporation • 5791 Van Allen Way • Carlsbad, CA 92008 • Phone: 760 603 7200 • FAX: 760 602 6500
www.lifetechnologies.com

Features of pJTI™ R4 Exp CMV EmGFP pA

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

Feature	Benefit
CMV promoter	Human cytomegalovirus immediate-early (CMV) promoter/enhancer for high-level expression in a wide range of mammalian cells
EmGFP	cDNA for Green Fluorescent Protein (Emerald) allows detection of retargeted cells by fluorescence microscopy or flow cytometry
HSV TK polyA signal	The Herpes Simplex Virus thymidine kinase polyadenylation signal for proper termination and processing of the recombinant transcript
EF1α promoter	Drives the high level expression of the Blasticidin resistance gene following the retargeting into the genomic R4 site of the parental Jump-In™ cell line
R4 *att*B site	Allows R4 integrase-mediated integration into the complementary genomic R4 *att*P site in the parental Jump-In™ cell line
Ampicillin resistance gene	Allows the selection and propagation of the vector in *E. coli*

Propagating pJTI™ R4 Exp CMV EmGFP pA

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

Materials Required but Not Provided

The table below lists ordering information about the competent *E. coli* cells that you can use to propagate vectors. For more information, refer to protocol supplied with the competent cells (also available at **www.lifetechnologies.com**) or contact Technical Support.

Product	Quantity	Cat. no.
One Shot® ccdB Survival™ 2 T1^R Competent Cells	10 reactions	A10460
One Shot® Mach1™ Phage-Resistant Chemically Competent *E. coli*	20 × 50 µL	C8620-03
One Shot® TOP10 Chemically Competent *E. coli*	10 × 50 µL	C4040-10

Purchaser Notification

Limited Warranty

Life Technologies Corporation is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about a Life Technologies product or service, contact our Technical Support Representatives.

All Life Technologies products are warranted to perform according to specifications stated on the certificate of analysis. The Company will replace, free of charge, any product that does not meet those specifications. This warranty limits the Company's liability to only the price of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. The Company reserves the right to select the method(s) used to analyze a product unless the Company agrees to a specified method in writing prior to acceptance of the order.

Life Technologies makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore the Company makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives.

Life Technologies Corporation shall have no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Limited Use Label License: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Use Label License No. 19: Gateway® Cloning Products

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for profit entity). The purchase of this product does not convey a license under any method claims in the foregoing patents or patent applications, or to use this product with any recombination sites other than those purchased from Life Technologies Corporation or its authorized distributor. The right to use methods claimed in the foregoing patents or patent applications with this product for research purposes only can only be acquired by the use of ClonaseTM purchased from Life Technologies Corporation or its authorized distributors. The buyer cannot modify the recombination sequence(s) contained in this product for any purpose. The buyer cannot sell or otherwise transfer (a) this product, (b) its components, or (c) materials made by the employment of this product or its components to a third party or otherwise use this product or its components or materials made by the employment of this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the employment of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Notwithstanding the preceding, any buyer who is employed in an academic or government institution may transfer materials made with this product to a third party who has a license from Life Technologies under the patents identified above to distribute such materials. Transfer of such materials and/or information to collaborators does not convey rights to practice any methods claimed in the foregoing patents or patent applications. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Life Technologies Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that none of (i) this product, (ii) any of its components, or (iii) a method claim of the foregoing patents, was used in the manufacture of such product. Life Technologies Corporation will not assert a claim against the buyer of infringement of the above patents based upon the use of this product to manufacture a protein for sale, provided that no method claim in the above patents was used in the manufacture of such protein. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information on purchasing a license to use this product for purposes other than those permitted above, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200.

Gateway® Clone Distribution Policy

For additional information about Life Technologies corporation's policy for the use and distribution of Gateway® clones, see the section entitled Gateway® Clone Distribution Policy, next page.

Gateway® Clone Distribution Policy

Introduction

The information supplied in this section is intended to provide clarity concerning Life Technologies Corporation's policy for the use and distribution of cloned nucleic acid fragments, including open reading frames, created using Life Technologies Corporation's commercially available Gateway® Technology.

Gateway® Entry Clones

Life Technologies Corporation understands that Gateway® entry clones, containing *att*L1 and *att*L2 sites, may be generated by academic and government researchers for the purpose of scientific research. Life Technologies Corporation agrees that such clones may be distributed for scientific research by non-profit organizations and by for-profit organizations without royalty payment to Life Technologies Corporation.

Gateway® Expression Clones

Life Technologies Corporation also understands that Gateway® expression clones, containing *att*B1 and *att*B2 sites, may be generated by academic and government researchers for the purpose of scientific research. Life Technologies Corporation agrees that such clones may be distributed for scientific research by academic and government organizations without royalty payment to Life Technologies Corporation. Organizations other than academia and government may also distribute such Gateway® expression clones for a nominal fee ($10 per clone) payable to Life Technologies Corporation.

Additional Terms and Conditions

We would ask that such distributors of Gateway® entry and expression clones indicate that such clones may be used only for research purposes, that such clones incorporate the Gateway® Technology, and that the purchase of Gateway® Clonase® from Life Technologies Corporation is required for carrying out the Gateway® recombinational cloning reaction. This should allow researchers to readily identify Gateway® containing clones and facilitate their use of this powerful technology in their research. Use of Life Technologies Corporation's Gateway® Technology, including Gateway® clones, for purposes other than scientific research may require a license and questions concerning such commercial use should be directed to Life Technologies Corporation's licensing department at 760-603-7200.

LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

For research use only. Not intended for human or animal therapeutic or diagnostic use.