Certificate of Analysis

pTRE-CellCycle Vector

Catalog No.	Amount	Lot Number
631466	10 µg	Specified on product label.

Description

pTRE-CellCycle is a bidirectional, tetracycline (Tet)-inducible expression vector that lets you inducibly express fluorescent, ubiquitination–based, cell-cycle indicators (Fucci; Sakaue-Sawano et al. 2008) in order to monitor all phases of the cell cycle. The vector allows you to identify cells that are either in G1 phase or transitioning between S, G2, and M phases. pTRE-CellCycle is intended for use with any Tet-On® or Tet-Off® Expression System.

Package Contents

- 20 µl pTRE-CellCycle Vector (500 ng/µl)

Storage Conditions

- Store plasmids at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

Shipping Conditions

- Dry ice (–70°C)

Product Documents

Documents for our products are available for download at **takarabio.com/manuals**
The following documents apply to this product:

- Tet-On Advanced Inducible Gene Expression System User Manual (PT3898-1)
- Tet-Off Advanced Inducible Gene Expression System User Manual (PT3945-1)

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(041318)

Vector Information

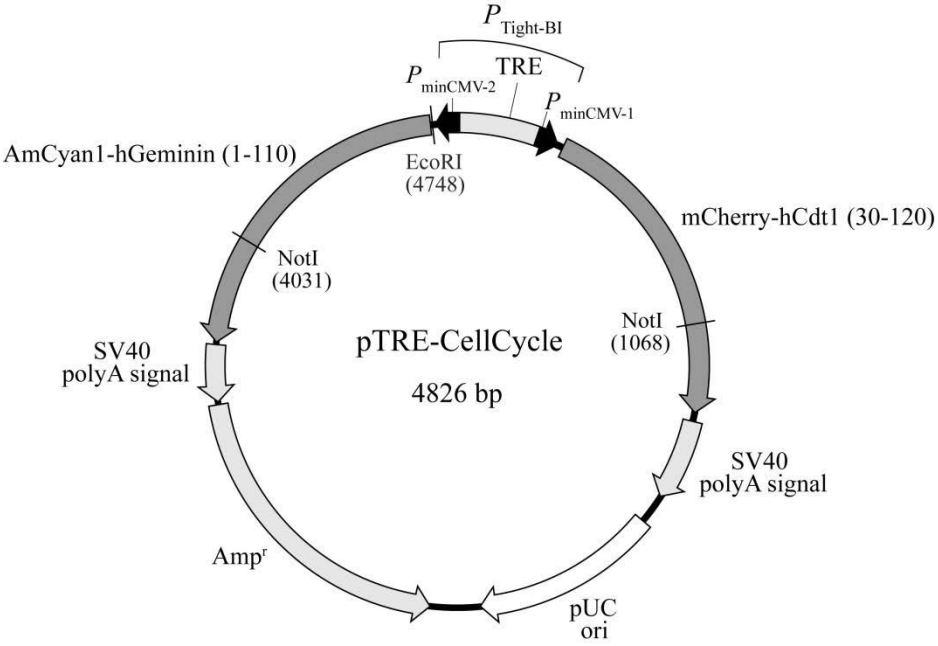

Figure 1. pTRE-CellCycle Vector Map.

Description

pTRE-CellCycle is a bidirectional Tet-inducible expression vector that can be used to inducibly express the cell cycle indicators hGeminin (1–110), tagged with the cyan fluorescent protein AmCyan1, and hCdt1 (30–120), tagged with the red fluorescent protein mCherry (McGarry and Kirschner 1998; Nishitani, Lygerou, and Nishimoto 2004). Both fusions can be induced simultaneously in our Tet-On and Tet-Off cell lines.

The AmCyan1-hGeminin (1–110) fusion is present at high levels during the S, G2 and M phases of the cell cycle, whereas the mCherry-hCdt1 (30–120) fusion is present at high levels during the G1 phase of the cell cycle. AmCyan1 is a human-codon optimized variant of the wild type Anemonia majano cyan fluorescent protein (excitation and emission maxima of 458 nm and 489 nm, respectively; Shaner et al. 2004), and mCherry is a mutant fluorescent protein derived from the tetrameric Discosoma sp. red fluorescent protein DsRed (excitation and emission maxima of 587 nm and 610 nm, respectively; Clontech 2003). Because both fusions are expressed together, the presence and absence of cyan and red fluorescence can be used to monitor all phases of the cell cycle.

Expression of both fusion proteins is tightly controlled by $P_{Tight-BI}$, a bidirectional, Tet-responsive promoter. PTight-BI consists of a tetracycline response element (TRE) flanked by two minimal CMV promoters (PminCMV-1 and PminCMV-2). The TRE is recognized and bound by a Tet-On or Tet-Off transactivator in the presence or absence of doxycycline (Dox), respectively (Clontech 2003; Gossen and Bujard 1992; Gossen et al. 1995). Thus, expression of both genes is responsive to the transactivators in the Tet-On and Tet-Off systems. pTRE-CellCycle should be cotransfected with the Linear Hygromycin Marker (Cat. No. 631625, not included) or Linear Puromycin Marker (Cat. No. 631626, not included).

Location of Features

- $P_{Tight-BI}$ (bidirectional, Tet-responsive promoter):
  - TRE (Tet response element): 3–252
  - $P_{minCMV-1}$ (minimal CMV promoter 1): 258–317
  - $P_{minCMV-2}$ (minimal CMV promoter 2): 4758–4826 (complementary)
- mCherry-hCdt1 (30–120) (mCherry and hCdt1 gene fusion): 341–1351
- SV40 polyA signal: 1374–1574
- pUC19 origin of replication: 1737–2336
- Amp^r (ampicillin resistance gene; β-lactamase): 2497–3493 (complementary)
- SV40 polyA signal: 3480–3683 (complementary)
- AmCyan1-hGeminin (1–110) (AmCyan1 and hGeminin gene fusion): 3694–4740 (complementary)

Additional Information

Dox-regulated expression of the proteins requires the presence of a tetracycline-controlled transcriptional activator, supplied by a stable Tet-On Advanced or Tet-Off Advanced cell line that can be created with our Tet-On Advanced or Tet-Off Advanced Inducible Gene Expression Systems (Cat. Nos. 630930 and 630934). These systems provide the inducible gene expression strategy of Gossen & Bujard (7, 8), with major improvements described by Urlinger, et al. (9, 10).

NOTE: Overexpression of the fusion proteins could lead to insufficient proteosomal degradation, which could prevent effective monitoring of the cell cycle. Therefore, we recommend either (i) optimizing gene transfer conditions to avoid overexpression of the fusion proteins, or (ii) selecting cells that express acceptable amounts of the fusion proteins.

Propagation in *E. coli*

- Recommended host strain: Stellar™ Competent Cells
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

Excitation and emission maxima of AmCyan1

- Excitation maximum = 458 nm
- Emission maximum = 489 nm

Excitation and emission maxima of mCherry

- Excitation maximum = 587 nm
- Emission maximum = 610 nm

References

Clontech. pTRE-Tight Vectors. **XVIII,** 13–14 (2003).

Gossen, M. *et al.* Transcriptional activation by tetracyclines in mammalian cells. *Science* **268,** 1766–9 (1995).

Gossen, M. & Bujard, H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. U. S. A.* **89,** 5547–51 (1992).

McGarry, T. J. & Kirschner, M. W. Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell* **93,** 1043–53 (1998).

Nishitani, H., Lygerou, Z. & Nishimoto, T. Proteolysis of DNA replication licensing factor Cdt1 in S-phase is performed independently of geminin through its N-terminal region. *J. Biol. Chem.* **279,** 30807–16 (2004).

Sakaue-Sawano, A. *et al.* Visualizing Spatiotemporal Dynamics of Multicellular Cell-Cycle Progression. *Cell* **132,** 487–498 (2008).

Shaner, N. C. *et al.* Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat. Biotechnol.* **22,** 1567–72 (2004).

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Enzymes	Fragment Sizes
EcoRI	4.8 kb
NotI	1.9 & 3.0 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

pTRE-CellCycle Vector

CATALOG NO.

631466

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Our products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Takara Bio USA, Inc.

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STATEMENT 24

The RCFPs (including DsRedExpress, DsRedExpress2, and E2-Crimson) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915; 6,969,597; 7,150,979; 7,442,522 and 8,012,682.

STATEMENT 44

The DsRed-Monomer and the Fruit Fluorescent Proteins are covered by one or more of the following U.S. Patents: 7,005,511; 7,157,566; 7,393,923 and 7,250,298.

STATEMENT 72

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4/13/2018

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STATEMENT 88

This product is sold under license from RIKEN and Tokyo Metropolitan Organization for Medical Research. This product is the subject of U.S. Patent No. 8,182,987 and its foreign counterparts.

STATEMENT 42

Use of the Tetracycline controllable expression systems (the "Tet Technology") is covered by a series of patents including U.S. Patent # 7541446, # 8383364, # 9181556 , European patents EP # 1200607, # 1954811, #2352833 and corresponding patent claims outside these regions which are proprietary to TET Systems GmbH & Co. KG. Academic research institutions are granted an automatic license with the purchase of this product to use the Tet Technology only for internal, academic research purposes, which license specifically excludes the right to sell, or otherwise transfer, the Tet Technology or its component parts to third parties. Notwithstanding the above, academic and not-for profit research institutions whose research using the Tet Technology is sponsored by for profit organizations, which shall receive ownership to any data and results stemming from the sponsored research, shall need a commercial license agreement from TET Systems in order to use the Tet Technology. In accepting this license, all users acknowledge that the Tet Technology is experimental in nature. TET Systems GmbH & Co. KG makes no warranties, express or implied or of any kind, and hereby disclaims any warranties, representations, or guarantees of any kind as to the Tet Technology, patents, or products. All others are invited to request a license from TET Systems GmbH & Co. KG prior to purchasing these reagents or using them for any purpose. Takara Bio USA, Inc. is required by its licensing agreement to submit a report of all purchasers of the Tet-controllable expression system to TET Systems.

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