Applied StemCell, Inc.
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Datasheet

Site-Specific Knock-in Technology
TARGATT™

TARGATT™ mESC Master Cell Line & Knock-in Kit

Product Information

Catalog Number

AST-7001

Size

One Kit (2 rounds of transfection)

Description

The **TARGATT™ mESC Master Cell Line & Knock-in Kit** is designed to create site-specific, knock-in in mouse embryonic stem cells (mESC) at a defined chromosomal locus in a fast, highly efficient way over traditional methods.

The kit includes (1) mESCs from C57BL/6 strain background mice (AST-0020) with a TARGATT™ docking site (attP) at a transcriptionally active locus (the H11 locus on chromosome 11)[1], (2) a plasmid for TARGATT™ integrase expression, and (3) a cloning vector to construct a donor DNA plasmid. With this method, large DNA fragments can be inserted at the H11 locus at high efficiency with robust transgene expression.

The TARGATT™ system takes advantage of the ΦC31 integrase enzyme from a Streptomyces phage. Integrases catalyze irreversible recombination between appropriate attB and attP sites[2]. The mESC Master Cell Line included in the kit have been engineered with attP sites inserted at the H11 locus, previously characterized to confer high level transgene expression and is in an intergenic region [2,3]. The DNA integration vector in the kit has the other ΦC31 integrase cognate site, attB. After transfecting the TARGATT™ mESC Master Cell Line with a vector expressing integrase and a DNA integration vector containing a gene of interest, recombination between attP site at the H11 locus and attB site on the integration vector results in transgene insertion at the H11 locus (Figure 1).

Application

Rapid generation of site-directed knock-in mESC lines.

Kit contents

All reagents supplied here are sufficient for up to 2 rounds of transfection according to the protocol below. Reagents are also available separately from Applied StemCell, Inc.

Shipping

Dry Ice

Shelf Life

Store the TARGATT™ integrase (AST-3100) and cloning vectors (AST-3043) at -20°C and avoid repeated freeze/thaw cycles. The TARGATT™ mESC Master Cell line (AST-0020) is stable for at least 6 months when stored in liquid nitrogen.

Limited Use Label License

This product is to be used for internal, non-commercial research purposes for the sole benefit of the purchaser. It may not be used for any other purpose, including, but not limited to diagnostics or therapeutics, and may not be used in humans. This product may not be transferred or sold to third parties, resold, modified for resale, or used to manufacture or develop commercial products or to provide a service of any kind to third parties, including, without limitation, reporting the results of purchaser's activities

for a fee or other form of consideration. For information on obtaining commercial research or additional rights, please contact Applied StemCell, Inc.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear appropriate Personal Protective Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling frozen vials. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

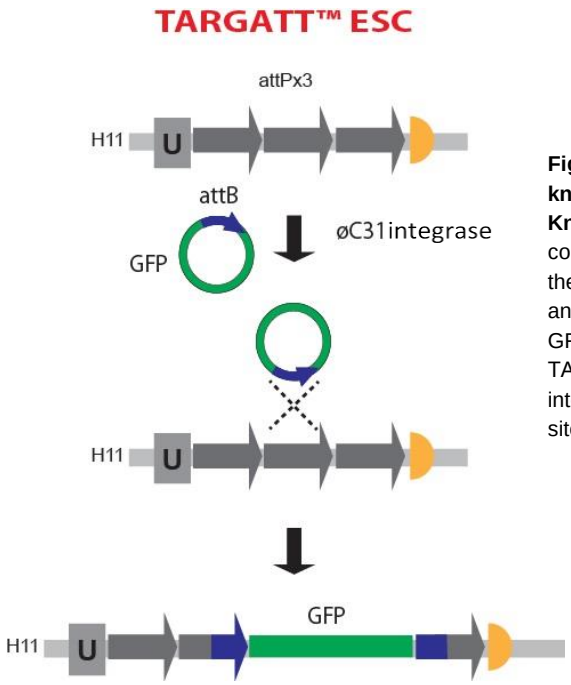

Figure 1. Scheme for site-specific gene insertion knock-in using TARGATT™ mESC Master Cell Line & Knock-in Kit. The TARGAT™ mESC Master Cell Line contains three tandem attP "docking" sites knocked into the H11 locus. A mix of the TARGATT™ integrase vector, and a donor vector containing the gene of interest (e.g., GFP) and an attB site was electroporated into the TARGATT™ mESC Master Cell Line. TARGATT™ integrase catalyzes recombination between attP and attB sites, resulting in integration of GFP.

Media and Material

Site-Specific Knock-in Technology TARGATT™

Knock-in Mouse Cell Line Generation kit

Cat. No.	Product Name	Conc.	Qty	Stability
AST-3100	TARGATT™ integrase vector	1 µg/µL	15 µL	Store at -20˚C. Avoid repeated freeze/thaw cycles.
AST-3043	TARGATT™ 3 cloning vector	100 ng/µL	20 µL	Store at -20˚C. Avoid repeated freeze/thaw cycles.
AST-0020	TARGATT™ mESC Master Cell Line (C57BL6)	1 X 10^6 cells/mL	1 vial x 10^6 cells	Store in gas phase of liquid nitrogen upon arrival.

Protocol

Transfection of TARGATT™ mESC Master Cell Line (Neon® Transfection System by ThermoFisher Scientific)

1. Prepare TARGATT™ mESC Master Cell Line and plate 1 x10^6 cells onto one well of 6-well plate 24 hours before electroporation.
2. Change medium in TARGATT™ mESC Master Cell Line two hours before electroporation.
3. Wash TARGATT™ mESC Master Cell Line with PBS.
4. Add 1 mL trypsin and incubate at 37°C for 5 minutes.
5. Neutralize trypsin with 5 mL of mESC medium and transfer cells to 15 mL conical centrifuge tube.
6. Centrifuge at 1,000 rpm for 5 minutes.
7. Aspirate the supernatant and resuspend the cells with 5 mL PBS.
8. Centrifuge at 1,000 rpm for 5 minutes.
9. Aspirate the supernatant and re-suspend the cells with 150 µL Buffer R.
10. Add 5 µg TARGATT™ Intergrase vector plus 10 ~ 20 µg TARGATT™ donor plasmid to the cells.
11. Carefully pipette the DNA/cells solution into NEON® 100 µL tip.
    Note: Avoid air bubbles
12. Insert the NEON® tip/NEON® pipettor complex into NEON® EP chamber containing the NEON® EP tube/E2 Buffer.
13. Electroporate the cell suspension using the following conditions:

    Pulse voltage: 1400 (v); Pulse width: 10 (ms); Pulse number: 3

14. Immediately after electroporation, pipette the cell suspension onto a 10 cm plate containing MEF and pre-warmed mESC media.

References

1. Tasic B, Hippenmeyer S, Wang C, Gamboa M, Zong H, Chen-Tsai Y, Luo L (2011). Site-specific integrase-mediated transgenesis in mice via pronuclear injection. Proc Natl Acad Sci U S A. [Epub ahead of print]
2. Thorpe HM, Smith MC (1998) In vitro site-specific integration of bacteriophage DNA catalyzed by a recombinase of the resolvase/invertase family. Proc Natl Acad Sci USA 95:5505–5510.
3. Hippenmeyer S, Youn YH, Moon HM, Miyamichi K, Zong H, Wynshaw-Boris A, Luo L (2010). Genetic mosaic dissection of Lis1 and Ndel1 in neuronal migration. Neuron 64(4): 695-709.