Applied StemCell, Inc.
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Datasheet

TARGATT™ CHO-K1 Kit (H11) for Antibody Library Screening (Drug Selection)

Product Information

Catalog Number

AST-1410

Description

The TARGATT™ CHO-K1 Kit (H11) for Antibody Library Screening (Drug Selection) was designed for fast and site-specific knock-in in Chinese Hamster Ovary (CHO) cells using an easy-to-use gene knock-in approach. The master cell line provided in this kit contains the "attP" docking-site engineered into the CHO H11 safe harbor locus in the genome. Any gene of interest can be cloned into the provided TARGATT™ "attB" cloning plasmid, and transfected into the master cell line for generating a stable knock-in cell line. The TARGATT™ integrase-based technology enables efficient DNA integration and high-level gene expression without disrupting internal genes. The TARGATT™ CHO-K1 cell line can therefore be used for uniform, site-specific gene knock-in, generation of isogenic cell lines, and amplification strategies for isolating high expression cells, without the need for single cell cloning.

Advantages of using TARGATT™ Master Cell Lines for gene knock-in:

- High efficiency, unidirectional integration
- Site-specific, stable knock-in cell line generation
- Single copy gene integration into safe harbor locus
- Gene expression from an active, intergenic locus
- Easy-to-use protocol requiring transfection of only one (donor) plasmid

The TARGATT™ CHO-K1 Kit (H11) for Antibody Library Screening (Drug Selection) includes the TARGATT™ 40 attB-Blas-P2A-LacZ (library) Cloning Plasmid (AST-3080) which contains blasticidinR. This kit is ideal for drug selection and is suitable for research applications involving gene library construction.

**TARGATT™ master cell lines can be generated in any cell line including stem cells. Please contact Applied StemCell for TARGATT™ cell line engineering services to generate a master cell line in a specific cell line of choice.*

**The TARGATT™ CHO-K1 Kit (H11) for Antibody Library Screening (FACS) is also available. The cloning plasmid in this kit contains mCherry and is ideal for fluorescence-activated cell sorting (FACS).*

Parental Cell Line

CHO-K1 Suspension Cells

Contents

The TARGATT™ CHO-K1 Kit (H11) for Antibody Library Screening (Drug Selection) contains the following:

- TARGATT™ CHO-K1 Master Cell line (AST-1408-1)
- TARGATT™ CAG Integrase Plasmid (AST-3203)
- TARGATT™ 40 attB-Blas-P2A-LacZ (library) Cloning Plasmid (AST-3080)
- TARGATT™ 25 Positive Control Plasmid (AST-3065)

Shipping

Dry ice

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Storage and Stability

Store TARGATT™ master cell line in liquid nitrogen freezer immediately upon receipt. Store the plasmids at -20°C. Do not freeze-thaw plasmids repeatedly. The products provided in this kit are stable for at least 6 months from the date of receiving when stored as directed.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protective Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. 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.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Schematic Representation of TARGATT™ Knock-in Strategy

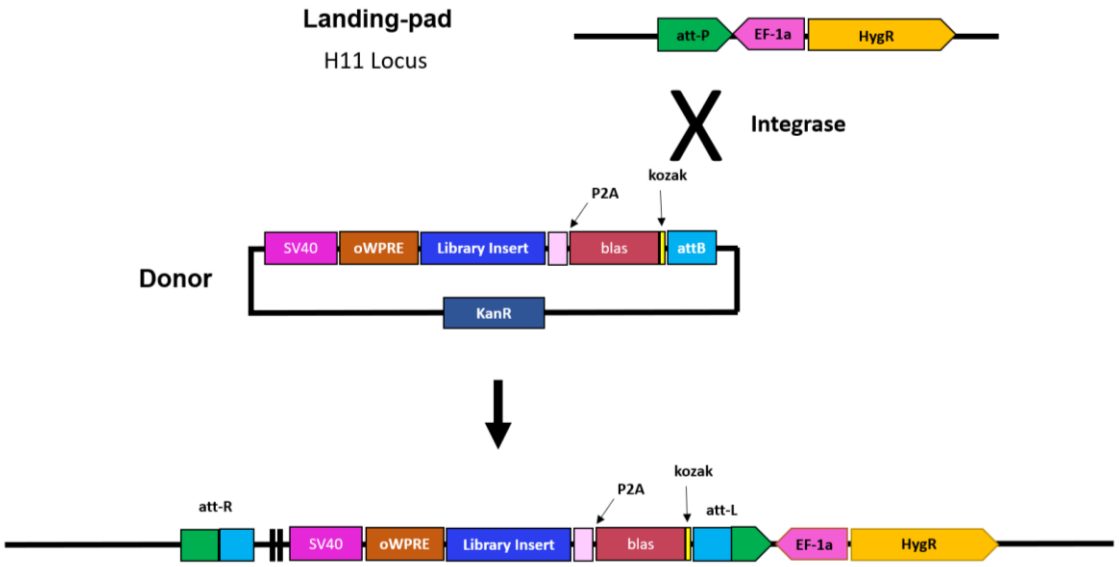

Figure 1. Schematic representation of TARGATT™ site-specific transgene integration mediated by integrase in the TARGATT™ CHO-K1 Master Cell Line. The TARGATT™ CHO-K1 (H11) Master Cell Line was engineered with the attP landing pad at the CHO H11 safe harbor locus. The TARGATT™ donor vector containing the attB site is used to clone the transgene. The integrase from the TARGATT™ Integrase Plasmid catalyzes an irreversible reaction between the attP site in the genome and attB site in the donor vector, resulting in site specific integration of the gene of interest at the selected locus.

Media and Material

Catalog#	Component	Amount
AST-1408-1	TARGATT™ CHO-K1 (H11) Master Cell Line	1x10^6 cells/vial
AST-3203	TARGATT™ CAG Integrase Plasmid#†	60 µg
AST-3080	TARGATT™ 40 attB-Blas-P2A-LacZ (library) Cloning Plasmid#	15 µg
AST-3065	TARGATT™ 25 Positive Control Plasmid#†	15 µg

 # Aliquot into single-use quantities and store at -20°C; do not freeze-thaw the plasmid repeatedly. † The positive control and integrase plasmids are proprietary materials and should not be amplified. Additional quantities of these plasmids are available for purchase.

Materials Needed but not Provided

Material	Vendor	Cat. Number
BalanCD® CHO Growth A Medium	Irvine Scientific	91128
L-Glutamine (200 mM)	ThermoFisher	25030081
PBS (10X), pH 7.4	ThermoFisher	70011
Dimethyl Sulfoxide (DMSO)	Sigma	D2438
Corning® CoolCell® LX, Cell Freezing Container	Corning	432000
Golden Gate Assembly Kit (BsaI-HF®v2) (20 or 100 reactions)	NEB®	E1601S E1601L
E. cloni ® 10G SUPREME SOLOs Electrocompetent Cells (12 or 24 transformations)	Lucigen	60081-1 60081-2
Recommended Systems for E. coli Electroporation Micro Pulser	Bio-Rad	165-2100
E. coli Pulser	Bio-Rad	165-2102
Gene Pulser II	Bio-Rad	165-2105
BTX ECM 630 Electroporation System		
Eppendorf Electroporator 2510		
Recommended Cuvettes for Electroporation of E. coli		
BTX (Model 610) Electroporation Cuvettes		
Eppendorf Electroporation Cuvettes		940001005
Electroporation Cuvettes	Bio-Rad	165-2089
Neon™ Transfection System	ThermoFisher	MPK5000
Blasticidin	InvivoGen	ant-bl-05

Protocol

1. Preparation of Medium

CHO Culture Medium (Complete Culture Medium)

- BalanCD® CHO Growth A Medium
- L-Glutamine (8 mM)

Cryopreservation/ Freezing Medium

- CHO Culture Medium (90%)
- DMSO (10%)

2. Thawing and Culturing Cryopreserved CHO-K1 Cells

2.1 Aliquot 30 mL CHO culture medium into a 50 mL conical tube, and warm in a 37°C water bath for 10 minutes. Before transferring into the hood, wipe the tube with paper towel and again with paper towel sprayed with 70% ethanol to disinfect.
2.2 Retrieve a vial of frozen TARGATT™ CHO-K1 master cell line from the liquid nitrogen tank wearing a thermal glove.
2.3 Immediately immerse the vial in a 37°C water bath. While holding the top of the vial, gently agitate the vial.
Note: Gently agitate the vial to thaw the cells quickly; DO NOT keep it static while in the water bath; DO NOT submerge the cap.
2.4 When almost completely thawed (usually takes ~1 minute, a small piece of ice is still visible), remove the vial from the water bath, and decontaminate the exterior of the vial with 70% ethanol.
Note: All further operations should be carried out under aseptic conditions.
2.5 Transfer the contents of the vial (1ml of cell suspension) to a conical tube containing 4.0 mL of pre-warmed complete culture medium.
2.6 Determine the viable cell count and calculate cell density, using an appropriate cell counter.
2.7 Centrifuge at **125 x g for 5 minutes.** Discard the supernatant.
2.8 Tap the tube to loosen up the cells and re-suspend the cell pellet in pre-warmed fresh complete culture medium at a density of 2.5 – 3.0 $x10^5$ viable cells/mL, in a fresh 125 mL Erlenmeyer shaker flask.
2.9 Place flask on an orbital shaker platform rotating at 130 rpm, in a 37°C and 5-8% CO_2 incubator.

3. Passaging Procedure

3.1 Once the culture reaches 2.5 – 5.0 $x10^6$ cell/mL, the cells are ready to be passaged.
3.2 Warm-up complete culture medium in a 37°C water bath for 10 minutes.
3.3 Take the 125 mL Erlenmeyer shaker flask out of the CO_2 incubator.
3.4 Determine the viable and total cell counts.
3.5 Seed the cells at a density of 2.0 – 3.0 $x10^5$ viable cells/mL into pre-warmed fresh culture medium by gently pipetting.
Note: Calculate the volume of cell culture suspension and fresh complete medium needed to seed each new flask by dilution.
3.6 Passage cells every 3-4 days.
3.7 Incubate cultures at 37°C and 5-8% CO_2.

4. Cryopreserving Cells

4.1 Harvest cells and transfer the contents of the vial into a centrifuge tube.
4.2 Centrifuge at 125 x g for 5 minutes.
4.3 Aspirate the supernatant and resuspend the cells in cryopreservation medium (refer to step 1).
4.4 Aliquot 1 mL of the cell suspension into cryovials and transfer the vials into a CoolCell® cell freezing container and store the vials into a -80°C freezer overnight.
4.5 Ensure cells are completely frozen and transfer the cryovials into liquid nitrogen for long-term storage.

5. Transfection: Fast Knock-in Procedure Using Neon™ Transfection System

Cloning with the TARGATT™ 40 attB-Blas-P2A-LacZ (library) Cloning Plasmid

5.1 Clone the gene of interest into the TARGATT™ 40 attB-Blas-P2A-LacZ (library) Cloning Plasmid to make the donor plasmid using the NEB® Golden Gate Assembly Kit (BsaI-HF®v2).
Note: See appendix for an example of how to design your cloning/library primers.

5.2 Amplify the plasmids using E. cloni® 10G SUPREME SOLOs electrocompetent cells.
Note 1: See the Lucigen E. cloni® 10G and 10GF´ Electrocompetent Cells manual for the transformation protocol.
Note 2: Do not use 5-alpha competent cells to transform the plasmids.

5.3 Purify plasmid pool, aliquot into single-use quantity, and store at -20°C. Do not freeze-thaw the plasmid repeatedly.

Preparation of Neon™ Transfection System

5.4 Aliquot a sufficient amount of Buffer E2 and Buffer R and warm at room temperature for at least 30 minutes.

5.5 Turn on the Neon™ Transfection System 30 minutes before use.

Cell Preparation for Electroporation

5.6 Pre-warm 6-well plates with 3 mL of fresh cell culture medium per well at 37°C.

5.7 Using cells already in the culture medium, count the cells and ensure >90% cell viability.

5.8 Harvest 1.1 x 10^6 cells and centrifuge the cells at 125 x g for 5 minutes.

5.9 Aspirate the supernatant and wash the cells by resuspending in 5 mL PBS.

5.10 Centrifuge the cell suspension at 125 x g for 5 minutes. Aspirate the supernatant.

5.11 Resuspend the cells by adding 110 µL Neon® Buffer R.

5.12 Aliquot a 110 µL cell suspension into a 1.5 mL Eppendorf tube and add 7.5 µg cloned TARGATT™ donor plasmid containing your gene of interest and 2.5 µg TARGATT™ CAG integrase plasmid).

5.13 Flick the tube a few times to mix them well.

5.14 Electroporate the cells based on the following condition: 1600V, 30 ms, 1 Pulse with Neon™ electroporation (o)r any other transfection method-of-choice.
Note: If using other transfection methods, please optimize your protocol accordingly.

5.15 After electroporation, plate the cells in one-well of 6-well-plate, and transfer the plate into an incubator at 37°C with 5-8% CO_2. More than one cell well can be done per round of electroporation in order to increase the library size.

5.16 At day 2-3 after electroporation, discard the spent medium and add 3 mL fresh, complete culture medium per well.

5.17 Place the culture plate containing the cells into an incubator at 37°C with 5-8% CO_2, on an orbital shaker platform rotating at 130 rpm.

6. Enrichment Using Blasticidin Selection

6.1 Seven to ten days after transfection when cell viability is above 90%, adjust the cell concentration to 1x10^6 cells/mL in 3 mL of culture medium in a 6-well plate.

6.2 Add 10 ug/mL of Blasticidin in the medium.

6.3 On day 3 of selection, harvest the cells by centrifuge at 125 x g for 5 minutes.

6.4 Remove the supernatant and resuspend cells in fresh culture medium with 10 ug/mL of Blasticidin.

6.5 Subculture the cells at a cell density of 1x10^6 cells/mL into a 6-well culture plate.

6.6 Monitor the cell viability daily during Blasticidin selection.

6.7 Keep Blasticidin in the medium for 5-7 days until cell viability is about 30% and then remove Blasticidin from the culture medium.

6.8 To stop the Blasticidin selection, harvest the cells and centrifuge the cell suspension at 125 x g for 5 minutes.

6.9 Remove the supernatant and resuspend the cells in fresh culture medium, and culture the cells in a 6-well plate until cells recover fully.

6.10 Passage the cells when the cell density is above 5x10^6 cells/mL.

Supporting Data

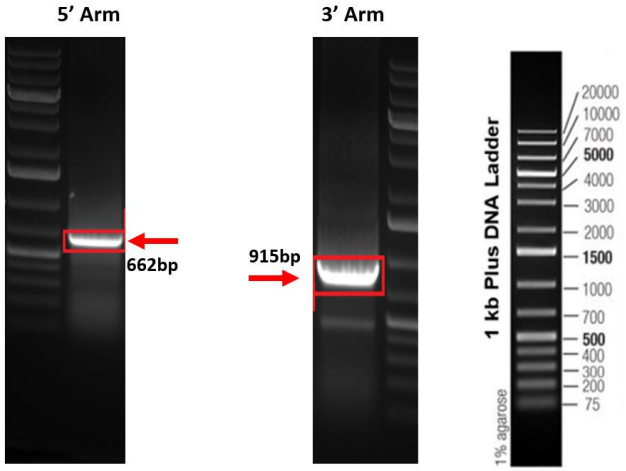

Figure 1. Genotyping of the 5'-arm and 3'arm in TARGATT™ CHO-K1 master cell line. PCR analysis shows the expected sizes for the 5' arm and 3' arm junction PCR product in a 1.5% agarose gel: 662bp and 915bp, respectively. Primer sequences used: 5'-arm-Forward: 5'-GGTTGATGACCCTGCACATAGC-3'; 5'-arm-Reverse: 5'-TGGATTTTGACTGCAGGGGTAAA-3'; 3'-arm-Forward: 5'-AGGTGTCTGCAGGCTCAAAGAG-3'; 3'-arm-Reverse: 5'-AGGCCTGGAGGTAGGTTTGTG-3'.

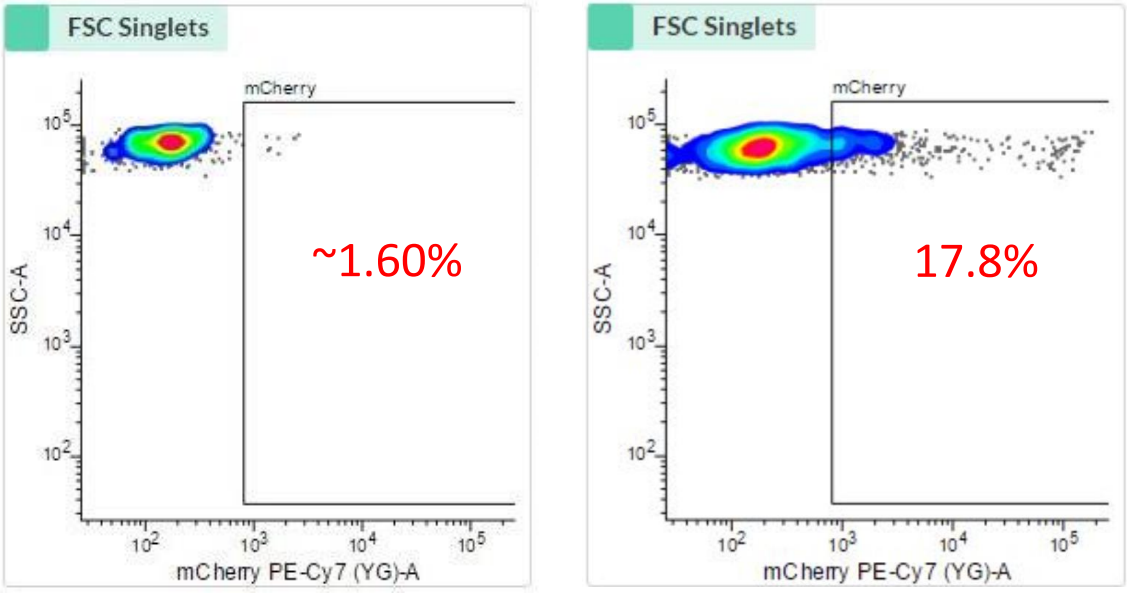

Figure 2. Flow cytometric analysis of mCherry expression level in CHO-K1 cells. A donor plasmid containing the fluorescent marker mCherry was used to analyze integration efficiency. An integration efficiency of ~18% without drug selection (right) and a negative control integration efficiency of ~1.60% (left) were obtained.

Appendix

Primer Design Example

These primers are for BsaI Golden Gate which require that your library not have a BsaI site. If necessary, alternative primers can be designed using other IIS restriction enzymes. If you choose to use other restriction enzymes, please contact Applied StemCell for assistance.

Primer	Sequence
Forward Primer	5' gtGGTCTCtTCCTatg ... 3'
*Where 'atg' is the start codon of your protein, and '...' is the rest of the primer that is specific to your coding sequence.	
Reverse Primer	5' caGGTCTCaTAAGcta ... 3'
*Where 'cta' is the reverse complement of the 'tag' stop codon, and '...' is the rest of the primer that is specific to the reverse complement of your coding sequence.	