Applied StemCell, Inc.
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Datasheet

TARGATT™ Human Induced Pluripotent Stem Cell (hiPSC, Female) Master Cell Line & Knock-in Kit

Product Information

Catalog Number AST-1602

Description

The TARGATT™ hiPSC Master Cell Line (Female) and transgenic kit were designed for fast site-specific knock-in in human iPSC cells, using an easy-to-use gene knock-in approach. The master cell line provided in this kit contains a dual-"attP" integrase recognition-site landing pad engineered into the H11 safe harbor locus in the genome. The kit also contains a cloning plasmid with corresponding "attB" sequences into which any gene of interest can be cloned (under control of the CAG promoter).

The expression of the integrase (provided as an integrase plasmid) mediates the stable integration of the transgene into the master cell line in a manner that excludes the bacterial plasmid backbone (Figure 1). The TARGATT™ integrase technology enables highly efficient site-specific integration after drug enrichment, without disruption of endogenous genes. The TARGATT™ iPSC master cell line and knock-in kit are ideal for transgene knock-in and uniform, stable expression of your protein*.

The TARGATT™ iPSC Master Cell Line (Female) has been engineered from our well-characterized, karyotype normal, control iPSC line, ASE-9209. The ASE-9209 parental iPSC line was reprogrammed using episomal factors from fibroblasts obtained with full consent from a 47-year-old, Caucasian female donor. Both the parental ASE-9209 line and the TARGATT™ iPSC Master Cell Line have been characterized for pluripotency biomarkers, normal karyotype, and directed differentiation to three germ layers as a validation of functional pluripotency.

The TARGATT™ iPSC master cell line and knock-in kit are suitable for research applications involving site-specific integration, large transgene knock-in, gene overexpression, variant library screens, and other stable cell line generation applications*.

**We also have TARGATT™ master cell lines in HEK293 and CHO backgrounds. TARGATT™ master cell lines can also be generated in any cell line. Please contact Applied StemCell for TARGATT™ cell line engineering services to generate a master cell line in a specific cell line of choice.*

Parental Line

Control human iPSCs (ASE-9209); p16
Age: 47
Gender: Female
Ethnicity: Caucasian
Tissue Source: Fibroblast
Reprogramming Method: Episomal
Culture Conditions: Feeder-free

Clinical information Healthy (with no known disease phenotypes)

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Contents

All cell lines and plasmids provided in this kit are sufficient for 3 transfections (i.e., 3 triplicate transfections) according to the given protocol:

- TARGATT™ hiPSC Master Cell Line (Female)
- TARGATT™ 45 CAG-MCS Cloning Plasmid
- TARGATT™ 44 mCherry-Blas Control Plasmid
- TARGATT™ CAG-Integrase Plasmid

Quality Control

A certificate of analysis (COA) with detailed quality control information for the cell line and components of the kit will be provided with each shipment.

Shipping

Dry ice

Storage and Stability

Store the 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 ™ Knockin Strategy

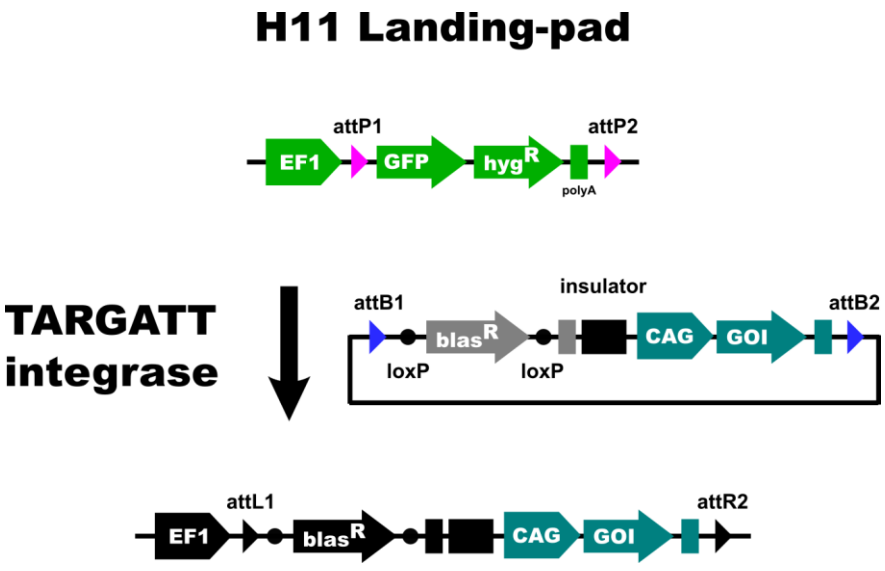

Figure 1. TARGATT-integrase mediated knock-in for stable expression of a gene of interest (GOI). The GOI donor plasmid is co-transfected with our provided integrase vector (not shown) to accomplish site-specific integration at the H11 safe-harbor locus. Cells where this reaction has occurred can be enriched by selection with blasticidin, and also by sorting for cells that have lost green fluorescence.

Media and Material

Catalog #	Component	Amount
AST-1602-C	TARGATT™ hiPSC Master Cell Line (Female)	1.0x10^6 cell/vial
AST-3086	TARGATT™ 45 CAG-MCS Cloning Plasmid*#	10 µg
AST-3084	TARGATT™ CAG-Integrase Plasmid#†	1.10 µg
AST-3085	TARGATT™ 44 mCherry-Blas Control Plasmid	3.31 µg

** The plasmids provided in this kit should not be amplified. Applied StemCell has a proprietary purification process that is necessary for high integration efficiency and health of the cells*

Materials Needed but not Provided

Material	Vendor	Catalog#
Rock Inhibitor Y-27632	Tocris	1254
CryoStor® CS10 Freeze Media Or CTS™ PSC Cryomedium	Stemcell Technologies ThermoFisher Scientific	210102 A4238801
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	ThermoFisher Scientific	A31804 A14700
OptiMEM | Reduced Serum Medium	ThermoFisher Scientific	31985062
Lipofectamine™ Stem Transfection Reagent	ThermoFisher Scientific	STEM00008
DPBS, No Calcium, No Magnesium	ThermoFisher Scientific	14190136
Essential 8™ Medium*	ThermoFisher Scientific	A1517001
Essential 8™ Flex Medium Kit*	ThermoFisher Scientific	A2858501
EmbryoMax® Nucleosides (100X)	Sigma-Aldrich	ES-008-D
TrypLE™ Express Enzyme (1X), No Phenol Red	Gibco	12604013
Versene Solution	ThermoFisher Scientific	15040066
Primocin, 50 mg/mL solution (100X)	InvivoGen	ant-pm-05
Blasticidin, 10 mg/mL solution (2000X)	Invivogen	ant-bl-05

*The above reagents are recommended based on our culture protocols. If you are using a similar/alternative reagent, we recommend that you perform a small-batch test using your preferred reagents. * Even if Essential 8 Flex is used for cell culture, regular Essential 8 is needed for transfection. As an alternative to Essential 8 Flex, thermostable bFGF can be added to regular Essential 8 (this does not interfere with transfection).*

Protocol

1. Thawing and culturing cryopreserved cells

To ensure the highest level of viability, thaw the vial containing the cells and culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1.1 Before thawing cells, coat two wells of a 6-well plate with VTN-N according to the manufacturer's instructions.
1.2 Prepare 9 mL of Essential 8 or Essential 8™ Flex media with 5 µM Rock Inhibitor and 0.5X EmbryoMAX Nucleosides, but without antibiotics ("E8+Ri").
1.3 Add 1.5 mL of E8+Ri to each coated well.
1.4 Use dry ice to bring one vial of frozen ASE-1602-C TARGATT™ hiPS cells to the cell culture room. Quickly thaw it in a 37°C water bath by gently shaking the cryovial continuously until only a small piece of ice remains.
1.5 Wipe the cryovial with a paper towel sprayed with 70% ethanol and place it in a biosafety hood.

1.6 Use a 5 mL serological pipette to transfer the cells into a 15 mL tube.
1.7 Slowly add 3 mL of E8+Ri in a dropwise fashion while swirling the 15 mL-conical tube.
1.8 Centrifuge the cells at 200 x g for 5 minutes.
1.9 Aspirate the supernatant and add 2 mL of E8+Ri media.
1.10 Gently flick the conical tube to resuspend the cells and then transfer 1 mL into each VTN-N coated well.
1.11 Place the plate in the incubator, then move it back-and-forth and side-to-side twice to spread the clumps evenly in the wells.

2. Sub-culturing procedure (culture maintenance)

2.1 The next day, aspirate the medium and add 2.5 mL of fresh Essential 8 or Essential 8™ Flex with 0.5X EmbryoMAX Nucleosides into each well. 1X Primocin can be included at this stage if desired.
2.2 Change the medium every day if using Essential 8, or on Mondays, Wednesdays and Fridays if using Essential 8 Flex (follow ThermoFisher's instructions).
2.3 When the colonies are big enough or close to merging, the cells need to be split.
Note: To ensure the best quality of cells, the cell culture should be passaged every 4-6 days.

2.4 Aspirate the medium from the culture wells.
2.5 Wash once with 1 mL of DPBS.
2.6 Aspirate the DPBS, add 1 mL of Versene per well, then incubate the cells for 3-5 minutes in a 37°C incubator.
2.7 Observe the cells under a microscope. When the cells at the edge of the colonies start to separate and round up, proceed to the next step.
2.8 With the cells still attached, aspirate the Versene and add 1 mL of Essential 8 or Essential 8™ Flex medium.
2.9 Pipette up and down 2-3 times to detach and break colonies into floating clumps. Do not over-pipette.
2.10 Split as desired (e.g., 1:4 – 1:15 ratio).
2.11 Place the plate in the CO_2 incubator and move the plate back-and-forth and side-to-side twice to spread the clumps evenly in wells.

3. Cryopreserving cells

3.1 Label the cryovials as needed, based on 2 vials per well of a 6-well plate.
3.2 Aspirate the medium from the hiPSC culture.
3.3 Wash once with 1 mL of DPBS.
3.4 Aspirate the DPBS and add 1 mL of Versene per well, then incubate the cells for 3-5 minutes in a 37°C incubator.
3.5 Observe the cells under a microscope. When the cells at the edge of the colonies start to separate and round up, proceed to the next step.
3.6 With the cells still attached, aspirate the Versene and add 2 mL of CryoStor® CS10 medium or PSC Cryomedium.
3.7 Pipette up and down 2-3 times to detach and break colonies into floating clumps. Do not over-pipette.
3.8 Aliquot the cell suspension in 2 cryovials: 1 mL in each vial.
3.9 Place the cryovials in a CoolCell® Freezing Container or in a Styrofoam rack at - 80°C overnight, then transfer to a liquid nitrogen storage tank the next day.

4. Transfection procedure – Lipofectamine Stem

Note: At least 30 minutes before cells are detached, add ROCK inhibitor (Ri) to wells that will be used. Also remember to coat wells with VTN-N (VTN), or to warm-up any pre-coated plates.

4.1 Label tubes that will contain the DNA mixes.
4.2 Label and prepare OptiMEM aliquots for Lipofectamine Stem (LS), but do not add LS yet (e.g., for transfection of 500k cells (single well of 6-well plate), aliquot 132 µL per tube).
4.3 Prepare wells:
4.3.1 In 24wp wells, replace VTN+DPBS with **200 uL Essential 8 + EmbryoMax Nucleosides (EMax) + Ri***
4.3.2 In 6wp wells, replace VTN+DPBS with **1mL Essential 8 + EMax + Ri***
**Note: Don't include hygromycin or any other antibiotic/selection drug when transfecting, and don't use Essential 8 Flex because it interferes with transfection.*

4.4 Centrifuge Trypan blue at max speed to pellet precipitates, then aliquot 10 µL per 0.2 mL tube that will be used.

4.5 iPSC harvest and count:
- 4.5.1 Wash iPSCs once with 1 mL DPBS, then add 0.75 mL TrypLE**
- 4.5.2 Incubate for 3-5 min at 37°C.
- 4.5.3 If the cells are still attached***, remove the supernatant, then add 1 mL Essential 8 + EMax + Ri (no hygromycin or any other selection drug).
- 4.5.4 Pipet thoroughly to detach and break-up cell clumps.
- 4.5.5 Centrifuge at 200g for 5 minutes.
- 4.5.6 Resuspend in Essential 8 +EMax + Ri medium and count.

***Note: If the colonies are big, use a 25% Versene 75% TrypLE mix instead.*

****Note: If they detached, add 0.75 mL TeSR+Ri, then pipet to mix and detach. Cell yield and viability might be lower.*

4.6 Pipet up and down to mix LS stock, do not vortex. Add LS to prepared aliquots and pipet to mix. E.g., for a single 500k cell transfection, add 5.50 µL LS to each 132 µL OptiMEM aliquot, then pipet up-and-down using an adequate volume to fully mix (50-100 µL).

4.7 Prepare DNA solutions. E.g., for a 500k cell transfection (single well of 6-well plate):
- 4.7.1 Combine 345 ng and 1035 ng of the integrase and donor plasmids, respectively.
- 4.7.2 Bring the total volume to 137.5 µL with OptiMEM.

4.8 Transfer full DNA solution into diluted LipoStem solution (e.g., for a 500k cell transfection, transfer 137.5 µL DNA into 137.5 µL diluted LS).
- 4.8.1 Mix by pipetting, do not vortex.
- 4.8.2 Incubate at room temp. for 10 minutes.

4.9 While LS complexes are forming, add cells to wells (e.g., 100k per well of 24-well; 500k per well of 6-well plate).

4.10 After the 10-minute incubation is finished, add DNA:LS complexes to cells. E.g., for a 500k cell transfection, transfer 250 µL of the complexes.

4.11 Bring the final volume of each 6-well well to 2.5 mL using Essential 8 + EMax + Ri. Mix using a figure 8 motion to evenly spread the cells in each well, and gently place the plate in your 37°C humidified CO2 incubator.

4.12 The next day, feed the cells with Essential 8 (or Essential 8 Flex) + EMax + Ri (no antibiotics).

4.13 Feed them again as needed. At this point, 1X Primocin (antibiotic) can be included.

4.14 Depending on how well the cells survived, it might be necessary to split them 2-4 days after transfection. Pre-treat the cells with Ri and use TrypLE to detach.

4.15 Four days after transfection, start blasticidin selection (5 ug/mL final concentration) and maintain it for 2 weeks. Include EMax + Ri, Primocin is optional. When colonies have started to form, stop including Ri.

Note: Always passage cells into wells with freshly coated VTN-N after 7-10 days or they will begin to detach.

4.16 Further enrichment can be performed via sorting for GFP-negative cells (as single cells or pools).

4.17 If desired, Cre recombinase can be used to remove the blasticidin marker (see appendix).

Appendix

Removal of blasticidin-resistance marker

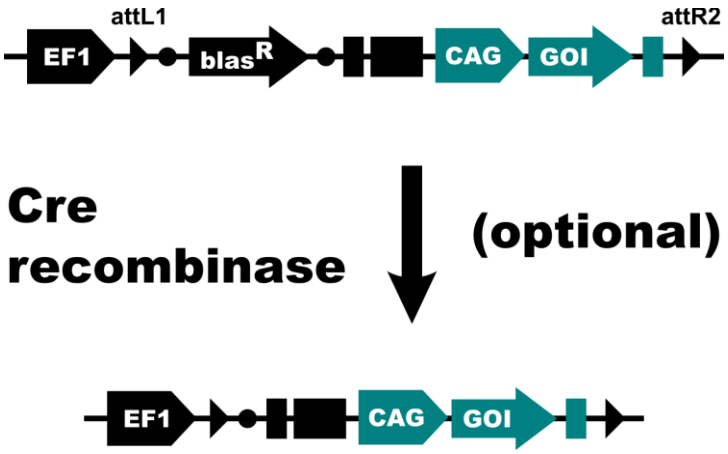

Figure 2. Cre-recombinase mediated excision of the blasticidin-resistance (blasR) marker. The blasR sequence can be removed from cells by exposing them to Cre recombinase – e.g. via the temporary addition of recombinant endotoxin-free Tat-Cre protein to the culture medium, or transfection of a Cre-expression plasmid, etc.

Primer Information

The following PCR method was used to perform the genotyping analysis to confirm integration of the AST-3086 plasmid:

(i) Set up PCR reaction on ice according to manufacturer's instruction for the *Taq* polymerase being used. Below is an example for PCR solution using the Promega GoTaq® Master Mix:

Component	Amount
Nuclease-free water	8.50µL
2X Promega GoTaq® Master Mix	12.50 µL
10µM 5junction F primer	1.00 µL
10µM 5junction R primer	1.00 µL
gDNA	2.00 µL
Total Volume	**25.00 µL**

(ii) Primer set: Please see Figure 1 (schematic) for location of primers

Junction Primers:	Forward Reverse	ggggaggggttttatgcgat CTGGCGACGCTGTAGTCTTC	387 bps

Note: the reverse primer anneals in blasR, so this PCR will fail if Cre recombinase has been used successfully.

(iii) Perform PCR amplification using the following programs for each primer sets.

Step	# of Cycles	5'+3` Junction		5' Junction	
		Temp	Time	Temp	Time
1	1	95°C	30 s	95°C	3 min

2 (Main PCR)	40	95°C 60°C 72°C	15 s 15 s 60 s	95°C 60°C 72°C	30 s 30 s 30 s
3	1	72°C	5 min	72°C	5 min
4		4°C	Hold	4°C	Hold