Applied StemCell, Inc.
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Datasheet

TARGATT™ Transgenic Kit (Version 2)

Product Information

Catalog Number

AST-1003 (Size: 5 reactions)
AST-1004 (Size: 2 reactions)

Description

The TARGATT™ Transgenic Kit is designed to create site-specific knock-in transgenic mice at a defined chromosomal locus in a more efficient and significantly faster way over traditional methods. Generating transgenic mice by conventional methods (e.g. pronuclear microinjection or lentiviral injection) has following limitations, first of which is random insertion of the transgene. Random insertion of a transgene results in position effect where either the transgene is prone to silencing or endogenous gene expression is disrupted. Secondly, transgenes can be inserted as multiple copies, resulting in instability at the insertion locus (see Appendix).

Using our proprietary site-specific DNA integration system, TARGATT™, combined with our genetically engineered TARGATT™ mice (Charles River strain code 537 and 549) or embryos (ASC cat. #AST-0001, #AST-0002, #AST-0003, #AST-0004, #AST-0012, #AST-0013), you can generate your desired mouse models with guaranteed gene expression in as little as three months [1].

Shipping

Dry ice

Storage and Stability

See table. (** The entire kit can be stored at -80˚C.) Components provided in this kit are stable for 6 months from the date of receipt 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). Handle the frozen vials with due caution.

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.

Warranty

Applied StemCell is committed to providing only superior-quality research products. Our products are specifically intended for research purposes only, and are guaranteed to perform according to documented product specifications and recommendations. Applied StemCell will not hold responsibility if components and reagents other than those provided and recommended with the AST-1003/ AST-1004 TARGATT™ Transgenic Kits are used.

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Item	Amount	Concentration	Catalog No.	Storage & Stability**
TARGATT™ Integrase (mRNA)*	20 µL/vial (2 or 5 vials)	100 ng/µL mRNA in miTE Buffer	AST-1011-2	Store at -80˚C. Avoid repeat freeze/thaw cycles.
Control mRNA	20 µL	50 ng/µL mRNA in TE Buffer	AST-1012	Store at -80˚C. Avoid repeat freeze/thaw cycles.
Embryo-qualified miTE Buffer	1 mL	0.1 mM EDTA, 10mM Tris pH 7.5	AST-1014	Store at -20˚C

** Transgene integration tested. Each vial is good for one round of microinjection.*

Reagents required but not provided

- TARGATT™ DNA Plasmid, Applied StemCell, Cat#s AST-3042/ 3043/ 3047/ 3048/ 3050/ 3051)
- NucleoBond® Xtra Midi EF, Macherey-Nagel, Cat# 740420
- One Shot™ Stbl3™ Chemically Competent *E. coli*, ThermoFisher, Cat# C737303
- LB Broth (Miller), Sigma-Aldrich, Cat# L3522
- LB Broth with Agar, (Miller), Sigma-Aldrich, L3147
- SOC medium, Clontech, Cat# 636763
- Nuclease-Free Water (not DEPC-Treated), ThermoFisher, Cat# AM9937
- CutSmart® Buffer, NEB, Cat# B7204S
- Agarose tablets, Nuclease-free, BioLine, Cat# BIO-41027
- NucleoSpin® Gel and PCR Clean-up, Macherey-Nagel, Cat# 740609
- Sterile 0.2 µm syringe filters (e.g. Millipore, Cat #SLGV004SL)

Protocol

The reagents and kits recommended are based on Applied StemCell's optimized protocols for efficient use of this kit. If you are using equivalent reagents from other manufactures, we recommend optimizing the protocols in your laboratory prior to microinjection.

1. Transformation of TARGATT™ Plasmid DNA

Notes:

- *If starting material is plasmid, transformation is necessary.*
- *We recommend using Stbl3 or other Stbl E. coli for transforming TARGATT™ vector and sub-cloned expression constructs.*
- *Stbl cells are cultured at 30°C. Vigorous shaking of bacterial cultures is not recommended.*

Use One Shot™ Stbl3™ Chemically Competent *E. coli* cells and follow the protocol provided by the manufacturer. Transformed E. coli in SOC mixture can be diluted to 20X or 50X before plating 50 µL of the diluted culture and incubate overnight at 30°C.

2. Endotoxin-free Plasmid Preparation and Purification

Notes:

- *Keep all buffer cold/on ice. Except the Lys-EF and Neu-EF buffers.*
- *Avoid shearing force during the process after cell lysis.*
- *Follow the instructions as close as possible.*
- *When using vacuum to empty column do not allow column/membrane to dry. Use the stop cock to control the vacuum rate. Use the mini adapters to avoid contamination of the columns (discard the adapters after use).*

2.1 Inoculate 1 Stbl *E. coli* colony into 3 mL LB broth with antibiotics and culture for no more than 14 hours at 30ºC.
2.2 Inoculate 200mL LB broth containing antibiotics with 500µL of the fresh culture for 16 to 22 hours.
2.3 Follow manufacturer's instructions for plasmid preparation and extraction using the NucleoBond Xtra Midi EF

Kit (REF#: 740420) from Macherey-Nagel.

Note: To avoid clogging of the filter, make sure to have a homogeneous suspension of the precipitate by inverting the tube 3 times, directly before applying the lysate to the equilibrated NucleoBond® Xtra Column Filter. Allow the column to empty by gravity flow or by vacuum.

2.4 Dissolve the DNA pellet in an appropriate volume of endotoxin-free Buffer TE-EF/H20-EF.
2.5 Measure DNA concentration using a NanoDrop spectrophotometer; use the TE-EF buffer as blank.
2.6 The plasmid yield should be > 1 µg/µL with $A_{260/280}$ value between 1.8-1.9.

Note: Avoid extensive pipetting of the TARGATT™ plasmid. Use of a small aliquot is recommended. Store the rest of the plasmid on ice or at 4°C.

3. Quality Control Assays for TARGATT™ Plasmid

3.1 Supercoiled DNA and Confirming Insertion Fragment in TARGATT™ Plasmid

3.1.1 Run 300ng of uncut, linear (single cut, recommended to be inside the insert sequence), and fragmented plasmid DNA (multiple restriction cuts) on a 1% agarose TAE gel with wide wells.

- For uncut plasmid, mix 300 ng DNA and ultra-pure water to have a total volume of 20 µL
- For restriction enzyme digested plasmid preparations, mix 300 ng DNA, 2 µL Cutsmart®, 0.5 µL of selected enzyme(s), and ultra-pure water for a total volume of 20 µL.
  Note: Use 3 sets of restriction enzymes to also confirm plasmid integrity and insertion fragment.
- Mix gently by flicking the tubes.
- Spin down restriction enzyme digested DNA for 2-3 seconds and incubate for 2 hours at 37°C. Do not spin uncut plasmid.
- Add 3 µL loading dye to each sample.
- Load equal volume of samples in each well of the 1% gel.

3.1.2 Resolve samples using electrophoresis at 70V for 1-2 hours or until all three fractions (open circle; OC, linear; L and supercoiled; SC) are separated into clear bands.

Note: The supercoiled fraction (the lower as compared to the linearized plasmid) should be the predominant fraction (>70%) in uncut plasmid prep in order to achieve a high success rate of site-specific insertion. If the linear or open circle fractions are > 10%, do not use the plasmid prep and start over. The gel picture in Figure 1 gives an example of a supercoiled TARGATT™ plasmid.

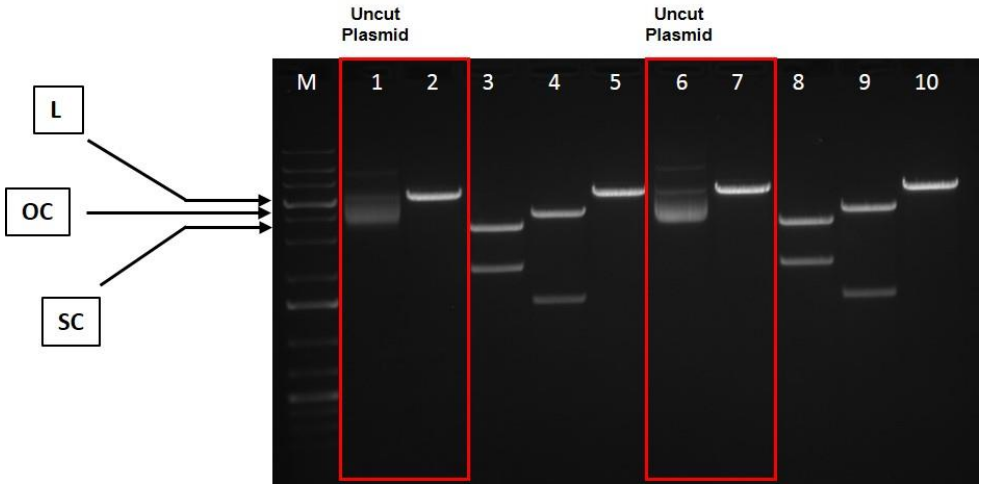

Figure 1. Representative gel image of a TARGATT™ Plasmid. Lanes 1 and 6 contain the uncut TARGATT™ plasmids; Lanes 2 and 7 depict the same plasmid in linear form. Both preps contain over 90% supercoiled form (lower band) and passed quality control. Lanes 3-5 and 8-10 represent three sets of restriction enzyme digest patterns, confirming the expected arrangement of the plasmid components.

3.2 Preparation of Microinjection Grade Plasmid DNA

Note: The following procedure requires ultra-precaution to avoid RNase contamination. All equipment and work surface should be treated with RNase away, or equivalent agents. Use frech gloves and change frequently.

3.2.1 Purify the plasmid DNA using NucleoSpin® Gel and PCR Clean-up PCR/ gel purification column. Follow manufacturer's instructions.

- To 15 µg of DNA, add ultra-pure water to make a final volume of 200 µL.
- Add 200 µL of binding buffer and load the entire volume onto a purification column.
- Spin at 11,000 x g for 1 minute.
- Discard the flow-through in the collection tube.
- Add 700 µL wash buffer to the column and spin at 11,000 x g for 1 minute and discard the flow-through. Repeat washing step once more.
- Spin one more time at 11,000 x g for 1 minute to completely remove all wash buffer.
- Elute the DNA from the column into a clean Eppendorf tube using 15 µL RNase-free TE buffer warmed at 55°C.
- Incubate at room temperature for 1 minute and centrifuge at 11,000 x g for 1 minute (elute 1).
- Repeat elution step using a new Eppendorf tube (elute 2).
- Measure DNA concentration and $A_{260/280}$ ratio for both elutes. If elute 1 has a concentration of >200 ng/mL, and a $A_{260/280}$ value of >1.8, proceed to confirm absence of RNase.

3.2.2 RNase-free test

- Incubate 200 ng of plasmid DNA (from elute 2) with 200 ng control RNA at 37°C for 1 hour.
- Spin samples briefly.
- Add 10 µL miTE buffer and 2µL 6x gel loading buffer. Incubate at 90°C for 5 minutes and cool down on ice for 5 minutes.
- Run the samples on a 1% agarose gel using a pre-chilled running buffer at 130V for 15 minutes. *Note: It is highly recommended to keep the gel-running system on ice using a clean gel box).*
- The mRNA band should be clear and sharp. There should be no RNA degradation shown as a smear on the gel.

4. Preparation of Samples for Microinjection

4.1 Dilute the purified plasmid DNA of elute 1 with sterile miTE buffer supplied with this transgenic kit to a final concentration of 10 ng/µL, and store it at -80°C till microinjection.

4.2 Mix equal volumes of TARGATT™ plasmid (10 ng/µL) and TARGATT™ integrase mRNA (provided in the kit), and filter the mixture through a sterile 0.2 µm syringe filter just before injection. The 20 µL reaction mixture is sufficient for injecting up to 200 embryos.

Important Notes:

- *Ensure that > 70% TARGATT™ donor plasmid is in supercoiled form.*
- *TARGATT™ donor plasmid should be used for microinjection within 2 months after preparation.*
- *Do not freeze-thaw TARGATT™ donor plasmid. Make and freeze usage-sized aliquots for one-time microinjection use.*

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. Hollis RP, Stoll SM, Sclimenti CR, Lin J, Chen-Tsai Y, Calos M (2003). Phage integrases for the construction and manipulation of transgenic animals. Reprod Bio Endocrinol 1:79.
4. Belteki G, Gertsenstein M, Ow DW, Nagy A (2003) Site-specific cassette exchange and germline transmission with mouse ES cells expressing phiC31 integrase. Nat Biotechnol 21:321–324.

Appendix

	TARGATT™ Technology	Random Transgenic	Traditional Knock-in
Site Specific	Yes	No	Yes
Expression Level	High	Variable or Silenced	Good
Stable Expression	Yes	Variable	Yes
Internal Gene	Intact	Could be Disrupted	Intact
Copy of Transgene	Single	Multiple	Single
Homologous recombination in ES cells	Not required	Not required	Required
Turnaround Time	~3 Months	>6 Months	>9 Months