OmniTag System

Cat # OTXXX

User Manual

Please see individual components for storage conditions

Version 1
10/24/2022

Contents

Product Description

SBI's OmniTag System streamlines tagging any endogenous genes at its C-terminus when used with OmniTag F0, F1 or F2 Frame Selector Vector and OmniTag Minicircle Donor of your choice.

- Universal Minicircle Donors that work for tagging any gene
- No need to prepare target-specific homologous DNA constructs
- Foreign-DNA free, ready-to-use Minicircle Donors
- Frame Selector vectors to specify the insertion reading frame
- CMV or EF1alpha promoter options to express Cas9 from Frame Selector
- Simplicity and modularity at high efficiency - >90% tag-positive cells following antibiotic selection
- Multiplex- sequentially tag multiple genes in same cells with choice of appropriate donors
- Alleviate the requirement for protein-specific antibodies for endogenous gene expression quantification, protein localization or immuno-precipitation studies.

Possible Applications

Tagging endogenous genes at their C- terminus by editing the genomic locus opens up plethora of possible applications and offers advantages to researchers in various areas of research.

- Tagging with Luciferase (T2A-Gaussia Luciferase, NanoLuc) for expression measurement
- Tagging with various fluorescent proteins (TagGFP2, TagBFP, TagRFP and T2A-TurboGFP-PEST) for cellular localization and FACS (Fluorescence-Activated Cell Sorting)
- Tagging with small epitope tags (HA, Myc, Strep tag II, Streptavidin-binding peptide, AviTag, SpyTag, SunTag) for sensitive detection or affinity purification approach
- Tagging with a controllable destabilization tag (ProtoTuner DD) to achieve switchable gene expression
- Tagging with split tags (split-TEV protease) to assess protein-protein interaction

Why Choose OmniTag System?

Tagging endogenous genes at their C- terminus by editing the genomic locus meets with the challenge of removing stop codon and in frame editing so that the tag is fused and translated along with the protein of interest. SBI's OmniTag System addresses these challenges to simplify C-Terminal tagging of any endogenous gene.

OmniTag Minicircle Donors are minicircles DNAs that will be fully integrated at the genomic locus and offer important features such as

- Foreign DNA-free- no bacterial plasmid DNA sequences so low immune response
- More efficient transfections due to the small size- more copies per ug of DNA

List of Components

Catalog number	Product Name	Quantity
OT100-F0	OmniTag F0 Frame Selector Vector with CMV-hspCas9	10 µg
OT101-F1	OmniTag F1 Frame Selector Vector with CMV-hspCas9	10 µg
OT102-F2	OmniTag F2 Frame Selector Vector with CMV-hspCas9	10 µg
OT200-F0	OmniTag F0 Frame Selector Vector with EF1alpha-hspCas9	10 µg
OT201-F1	OmniTag F1 Frame Selector Vector with EF1alpha-hspCas9	10 µg
OT202-F2	OmniTag F2 Frame Selector Vector with EF1alpha-hspCas9	10 µg
OT501MC-1	copGFP-T2A-Puro-pA OmniTag Minicircle Donor	10-µg
OT502MC-1	RFP-T2A-Blast-pA OmniTag Minicircle Donor	10 µg
OT503MC-1	6xHis-T2A-Puro-pA OmniTag Minicircle Donor	10 µg
OT504MC-1	3xFlag-T2A-Puro-pA OmniTag Minicircle Donor	10 µg

Storage

The vectors are shipped on blue ice and should be stored at -20°C. Avoid repeated freeze-thaws of the minicircle donors.

How It Works:

OmniTag System is composed of 3 key components.

1> An all-in-one Cas9/gRNA vector, similar to SBI's CAS7XXX-1, to introduce a double stranded break at the genomic locus just upstream of stop codon of the gene of interest.

2> One of the three OmniTag Frame Selector Vectors that is also an all-in-one vector that contains Cas9 and specific gRNAs against frame selector linker in the OmniTag Minicircle Donor at one of the three possible positions (F0, F1, F2) to achieve in-frame fusion of the tag.

3> An OmniTag Minicircle Donor that is a minicircle DNA containing a frame selector linker, desired tag sequence and a selection marker separated by T2A which gets fully incorporated at the genomic locus by NHEJ after linearization at the frame selector linker

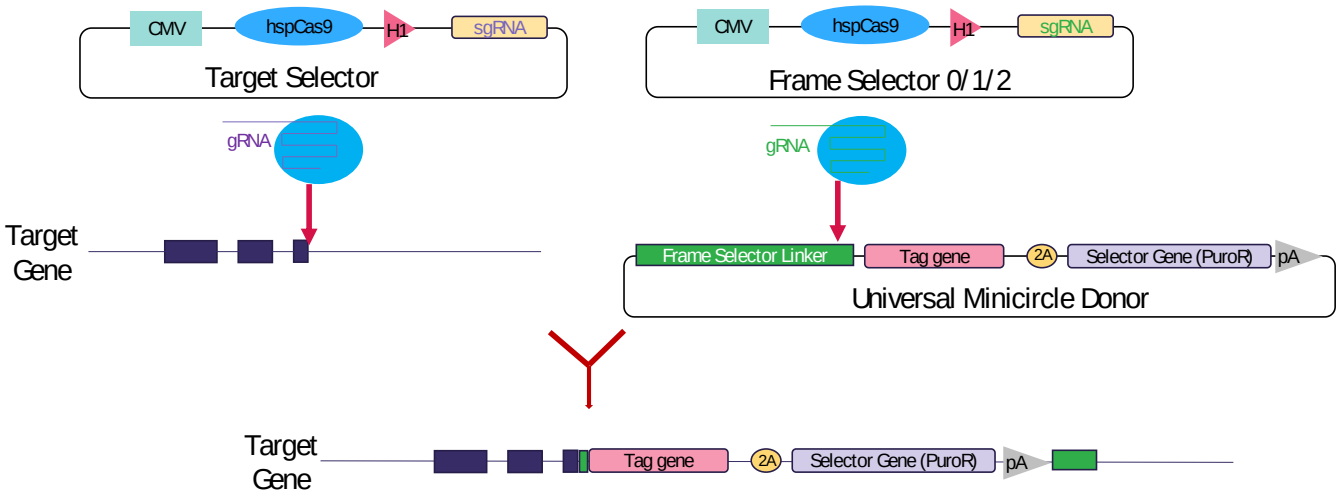

Figure 1. C Terminal tagging an endogenous gene at its genomic locus using OmniTag System

The Workflow At-A-Glance

CHOOSE: target gRNA and OmniTag Frame Selector

To simplify the application of C-terminal tagging using OmniTag System, a list of pre-selected, sequence-optimized target sites close to translation termination codons of nearly all human and mouse protein coding genes in conjunction with the required Frame Selector can be found here.

For human gene: human: https://www.systembio.com/wp/wp-content/uploads/2022/10/human-1.xlsx

For mouse genes: mouse: https://www.systembio.com/wp/wp-content/uploads/2022/10/mouse-1.xlsx

CONSTRUCT: Clone gRNA into all-in-one Cas9 vector such as CAS7XXX-1 as Target Selector

CO-TRANSFECT: Introduce Cas9, target gene gRNA, OmniTag Frame Selector Vector and OmniTag Minicircle Donor of your choice into the target cells using co-transfection

SELECT/SCREEN: Select or screen clones for verification.

VALIDATE: Genotype/Fluorescent microscopy/Western Blot/Sequencing.

Protocol for C-Terminal Gene Tagging with OmniTag System

General Comments

We recommend propagation of the Frame Selector plasmids prior to starting the experiments. The plasmids can be transformed using standard conditions suitable in any E. coli competent cell. Cells with the Frame Selector vectors should be grown on LB-Kanamycin plates (50μg/ml). Incubate the plates at 37°C overnight. Colonies picked from the transformation can be grown at 37°C overnight in ~200ml of LB media containing Kanamycin. After overnight growth, plasmid DNA can be harvested from culture using an endotoxin-free DNA plasmid maxiprep kit. For confirmation of the plasmid, we recommend performing restriction digestion analysis or direct sequencing to confirm integrity of the amplified plasmids.

Co-transfection of Target Selector, Frame Selector and OmniTag Minicircle Donor

1. Plate ~1-2x10^5 cells (e.g., 293T cells) into single well of a 12-well plate in 1 ml of appropriate growth medium. Include wells for the following:
    a) Target Selector + Frame Selector + OmniTag Minicircle Donor

b) Frame Selector + OmniTag Minicircle Donor

2. Next day, or when cells are 50-60% confluent, prepare transfection complexes of Target Selector + Frame Selector + OmniTag Minicircle Donor plasmids using a suitable transfection reagent such as Lipofectamine 2000 reagent according to the manufacturer's recommended instructions. Leave the transfection complex on the cells for >6 hours.

Example: For HEK293T cells using Lipofectamine 2000 reagent, transfect 0.5 µg of Target Selector, 0.5 ug of Frame Selector and 0.5 ug of OmniTag Minicircle Donor.

Tech Notes:

a) Since transfection efficiencies vary across different cell lines, we recommend optimizing the input of Target Selector, Frame Selector and OmniTag Minicircle Donor for best results. We recommend starting with a 1:1:1 ratio to 1:1:2 ratio.

b) For optimal results, we recommend complexing of DNA with transfection reagent in serum- and antibiotic-free media and cells growing in complete media (e.g., DMEM/F12+10% FBS+10% Glutamax w/o antibiotics).

c) For hard-to-transfect cells (e.g., primary, stem, hematopoietic), it may be advisable to utilize a nonpassive transfection method such as NucleoFection (Lonza) or Neon system (Life Technologies). Please follow recommended transfection guidelines provided by the manufacturer for specific cell type(s) being transfected.

3. 24 hours post-transfection, remove transfection media and plate cells into 6-well plates. Allow cells to recover for 24 hours.

4. Begin puromycin selection 2-4 days post-transfection, when cell confluence is roughly 60-70%. For 293T cells, the recommended concentration of puromycin is 0.5-1 µg/ml,

Tech Note:

The effective working puromycin concentration for a target cell line can be determined by establishing a kill-curve on untransfected cells. The concentration of puromycin (typical working range of 0.5µg-5µg/ml) that kills >90% of cells after 48h of selection is the correct dose for the cells being selected.

Characterization of Tagged Recombinant Cells

1. Check puromycin resistance.

a) Target Selector + Frame Selector + OmniTag Minicircle Donor: Select cells in Puromycin for 7-10 days, resulting colonies should be Puro resistant.

b) Frame Selector + OmniTag Minicircle Donor: Select cells in Puromycin for 7-10 days, very few colonies (if any) should be seen. Presence of $Puro^R$ colonies indicates frequency of random integration events.

2. Check fluorescent signal under fluorescent microscope, if tagging gene with fluorescent markers. Make sure the fluorescent signal is in line with the cellular localization of the target gene.

3. Confirmation of donor integration specifically at the target locus can be performed using junction and insertion PCR.

4. Check tagged gene expression using western blot, if tagging a gene with epitope-tags.

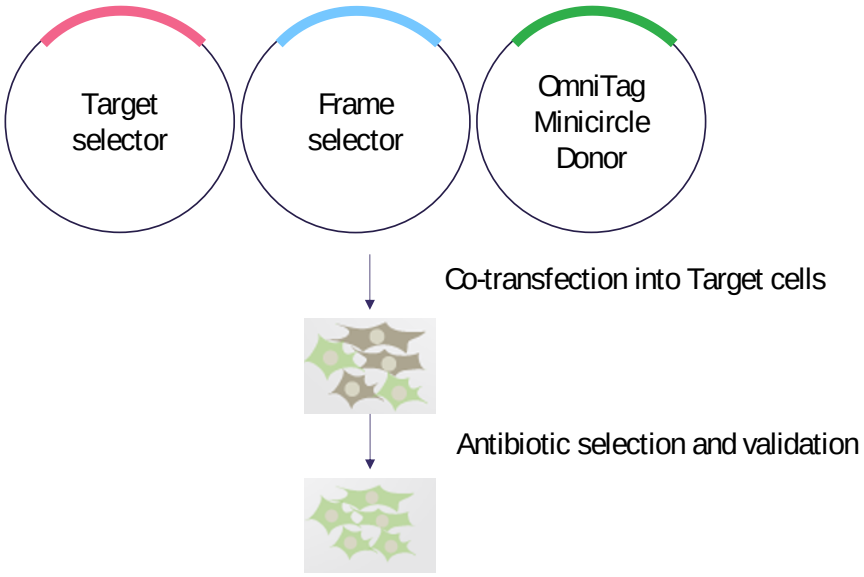

Figure 2. Workflow of OmniTag system for selection-based gene tagging in target cells.

Related Products

Cloning of gRNA for Target Gene

Product	Cat#	Website links
All-in-one gRNA-Cas9 SmartNuclease™ Plasmids	CAS7XXX-1	https://www.systembio.com/wp/wp-content/uploads/2020/10/gRNA_CAS7xx_20web-1.pdf

Example Data and Applications

Tagging of endogenous TUBB with GFP

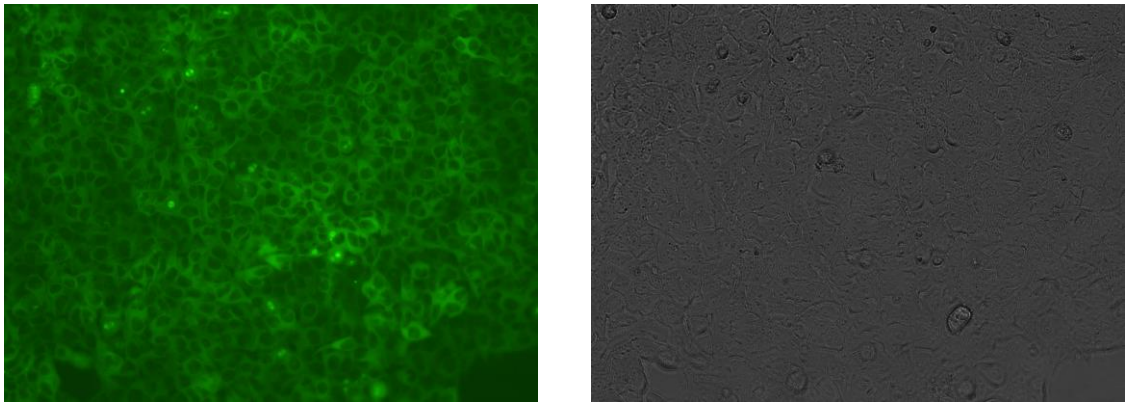

Figure 3. Using OmniTag System to tag endogenous TUBB. HEK293 cells were transfected with Cas9 all-in-one vector containing gRNA targeting TUBB, F1 Frame Selector and OmniTag GFP-Puro Minicircle Donor. 2-4 days after transfection, puromycin at 1μg/ml concentration was applied to cell culture medium. After 4-7 days, puromycin positive clones were obtained. Fluorescent microscope reveals the GFP tagging of TUBB with corresponding cellular localization.

Confirmation of precise integration of OmniTag Minicircle donor

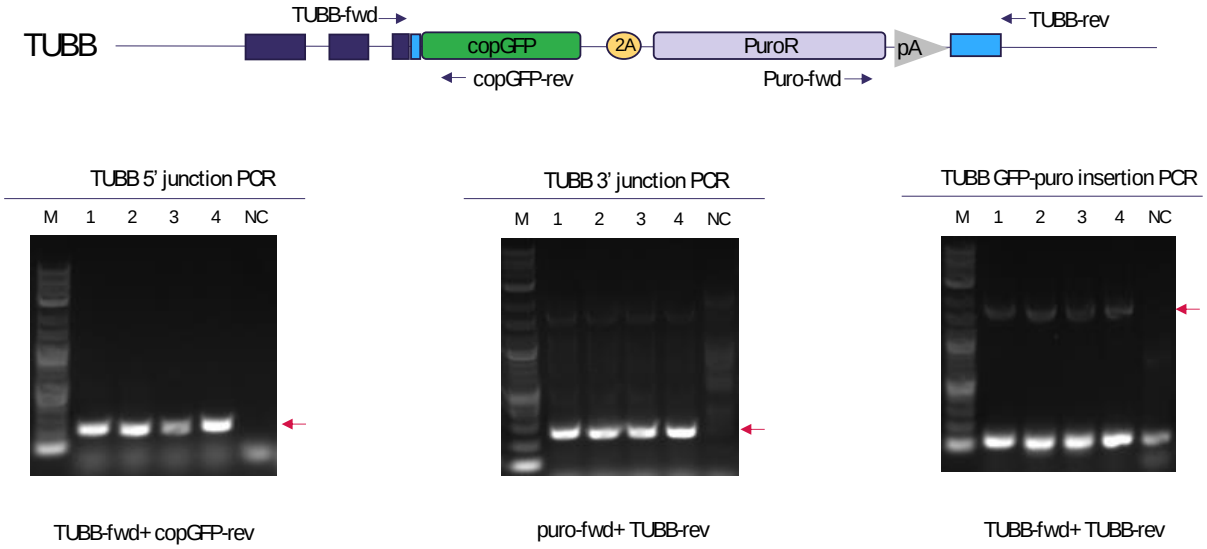

Figure 4. Verification of precise tagging with OmniTag System by junction and insertion PCR. Junction and insertion PCR were performed to confirm the correct integration of OmniTag MinicircleDonor at the TUBB target site. After puromycin selection, individual clones were expanded, followed by genomic DNA extraction and junction PCR. TUBB-fwd and copGFP-rev primers were used for 5' junction PCR, puro-fwd and TUBB-rev primers were used for 3' junction PCR. TUBB-fwd and TUBB-rev primers were used for minicircle donor insertion at TUBB target locus. Gel electrophoresis reveals all the clones contain the expected amplicon, indicating the precise insertion of the OmniTag minicircle donor at the desired TUBB target site.

Dual Tagging of H4C3 and TUBB with different fluorescent tags sequentially

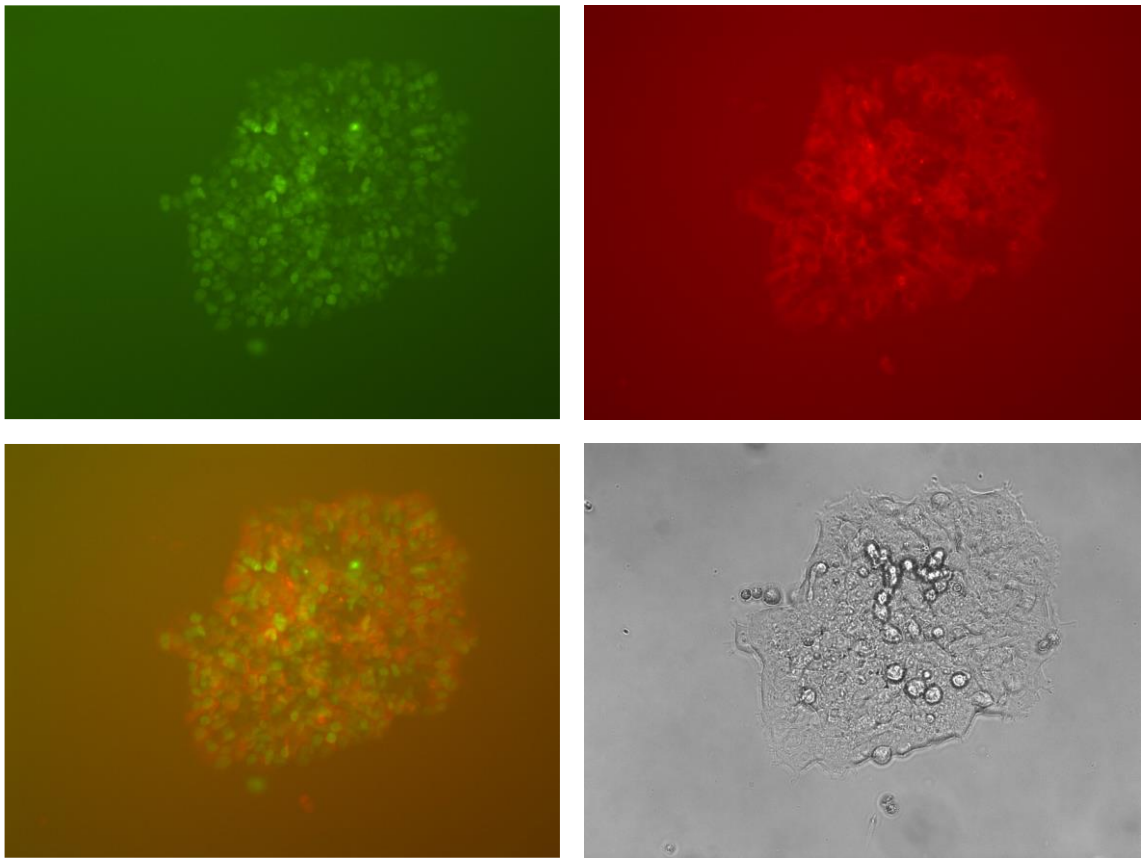

Figure 5. Dual tagging achieved by OmniTag system. HEK293 cells were first transfected with Cas9 all-in-one vector containing gRNA targeting H4C3, F1 Frame selector and OmniTag GFP-Puro minicircle donor. 2-4 days after transfection, puromycin at 1ug/ml concentration was applied to cell culture medium. After 4-7 days, puromycin positive clones were obtained. Then HEK293 H4C3-GFP tagged cells were further transfected with Cas9 all-in-one vector containing gRNA targeting TUBB, F1 Frame Selector and OmniTag RFP-Blast Minicircle Donor. 2-4 days after transfection, blasticidin at 7ug/ml concentration was applied to cell culture medium. After 4-7 days, blasticidin positive clones were obtained. Fluorescent microscope revealed the RFP tagging of TUBB and GFP tagging of H4C3 with corresponding cellular localization.

References

Jonathan L. Schmid-Burgk, Klara Höning, Thomas S. Ebert & Veit Hornung. CRISPaint allows modular base-specific gene tagging using a ligase-4-dependent mechanism. Nat Commun. 2016 Jul 28;7:12338. doi:10.1038/ncomms12338. PMCID: PMC4974478

Technical Support

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